RUMINAL MOTILITY AND BLOAT POTENTIAL OF

WHEAT PASTURE STOCKERS

By

DENZIL FAY FROST

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Purdue University

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Thesis Approved:

Derald W. Horn
Thesis Adviser

FM Owens

Lavoy I. Croy

Norman N Durham
Dean of the Graduate College

1011886

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CHAPTER I

INTRODUCTION

In years favorable to the growth of winter wheat pasture, more
than 1.5 million stocker cattle are grazed on Oklahoma wheat pastures.
The average stocking rate is about one steer per 6 hectares, and the
usual wheat pasture grazing period is from November 1 to March 15. With
grazed-out programs, the grazing period is extended to about May 31.
Stocker weight gains of .57 to .80 kg per day are common, indicating
the high quality of wheat forage.

Among the health problems of wheat pasture stockers is one known
as the stocker syndrome, or the sudden death syndrome. Previous studies
(Clay, 1973; Horn et al., 1974) have indicated that death due to the
stocker syndrome cannot be attributed to nitrate toxicity, acute
mineral imbalance, or clostridial toxin. High crude protein values
(14 to 33% of dry matter) have been reported for wheat forage
by Clay et al. (1972) and Horn et al. (1974). Johnson et al. (1974)
further reported that 17 to 33% of the total forage nitrogen is
in the form of non-protein nitrogen. These data along with high
ruminal ammonia concentrations and high ruminal pH values (6.2 to 7.6;
Horn et al., 1974), have prompted the suggestion that ammonia toxicity
may be an etiological factor in the sudden death syndrome. However,
Clay (1973) and Horn et al. (1975) have provided data to indicate that
the blood ammonia concentrations of stocker cattle on winter wheat

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forage per se were not high enough to result in ammonia toxicity. Coombe et al. (1960) observed that drenching fasted sheep via ruminal cannulae with 5 to 25 g of urea (concentration in fluid not exceeding 6%) inhibited ruminal motility and elevated ruminal pHs (7.3 to 7.8). These observations were accompanied by increased ruminal ammonia concentrations; however, signs of ammonia toxicity were not observed.

Specific signs of the sudden death syndrome led Clay (1973) to conclude that frothy bloat was a major cause of death in stocker cattle grazed on wheat forage. This conclusion was based on (1) observations of live stocker cattle exhibiting marked ruminal distension, (2) the nature of ruminal contents from dead animals, (3) the fact that some distended live animals were later found dead, and (4) necropsy lesions indicative of antemorteum bloat.

Plant chemical components have been postulated to contribute to the production of stable ruminal foams necessary for frothy bloat. However, correlations between specific plant chemical components and the incidence of bloat have proved elusive and somewhat nebulous (Clarke and Reid, 1974).

With the identification of the pre-disposing factors of frothy bloat and their possible interactions with one another, wheat pasture stocker operators may be able to reduce the incidence of bloat. The primary objectives of these studies reported herein were to: (1) determine if the frothy bloat of wheat pasture stockers occurs secondarily to a reduced ruminal motility, (2) measure ruminal fluid foam stability, expansion and strength (as indices of bloat potential)

throughout the wheat pasture grazing season, (3) measure changes in

concentrations of wheat forage chemical components, believed to be re-

lated to the incidence of bloat, and (4) assess possible relationships

between ruminal fluid foam measurements and the concentration of

chemical components in wheat forage.

CHAPTER II

REVIEW OF LITERATURE

Ruminal Motility and Eructation

Contractions

Rumino-reticular motility patterns were observed as early as 1833.
Flourens (1833; reported by Hungate, 1966) used fistulated sheep to de-
tect contractions of the rumen and reticulum, and also reported cessa-
tion of the contractions when both vagus nerves were cut (Duncan, 1953).

The basic movements of the rumino-reticulum have been divided into
two regular cyclical sequences of contractions (Titchen and Reid, 1965;
Sellers and Stevens, 1966). The first, which occurs about every minute,
is concerned primarily with mixing of rumen contents and consists of a
bi-phasic contraction of the reticulum followed in succession by a mono-
phasic contraction of the dorsal ruminal sac and then by a monophasic
contraction of the ventral ruminal sac. The second type of contraction
is independent of the reticular contractions and occurs approximately
every two minutes. These contractions are frequently referred to as
β contractions and primarily involve the rumen and eructation. These
β contractions consist of a contraction of the dorsal sac followed by
a contraction of the ventral sac. With each sequence of the β con-
tractions the contents of the dorsal sac are forced to move ventrally,
after which the gas layer is then directed cranially towards the

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cardia, where eructation occurs. These two sequences of rumino-reticular contractions are basically medullary reflexes (Titchen and Reid, 1965; Titchen, 1968; Harding and Leek, 1971), with both the efferent and afferent nerve fibers traveling in the vagal trunks (Habel, 1956; Stevens and Sellers, 1956; Titchen and Reid, 1965; Leek, 1969b). However, there is not complete agreement on the cyclical movements of the rumino-reticulum due to the variety of methods employed and the inherent species variations that exist among ruminant animals (Sellers and Stevens, 1966).

Stages of Gas Movement During Eructation

Clarke and Reid (1974) categorized the movement of gas during eructation into the following stages: (1) Separation Stage: Bubbles form from gas that rise through the injesta and coalesce with the free gas cap present in the dorsal rumen, (2) Displacement Stage: The free gas cap moves cranially and downward towards the cardia, (3) Transfer Stage: The cardia opens and the gas passes out of the rumen into the esophagus; with the aid of a contraction of muscles in the ruminal wall, (4) Esophageal Stage: The gas passes quickly, with the accompaniment of a rapid contraction of the esophagus, up the esophagus and enters into the pharynx via the superior cervical esophageal sphincter, (5) Pharyngeo-pulmonary Stage: Due to closure of the nasopharyngeal sphincter, the gas enters the opening of the epiglottis and is forced into the pulmonary cavity where a large portion is absorbed and the remainder is exhaled with the following expiration.

Effect of Rumen Fill on Eructation Efficiency

Eructation, a seemingly unimportant physiological process in man and most animals, is of great importantce in ruminants. Several groups of workers (Dougherty, 1940; Dougherty and Habel, 1955; Dougherty and Meredith, 1955; Stevens and Sellers, 1956; Dougherty et al., 1958) have all contributed significantly to the understanding of eructation. Large quantities of gas are produced in the ruminant stomach by microbial digestion of simple and complex carbohydrates. Most of the gas is produced in the first two compartments, the reticulum and the rumen. Hungate et al. (1955) estimated that about 1.2 to 2.0 ℓ of gas are formed per minute in the rumen and reticulum of a 454.5 kg steer. Leek (1969a) demonstrated that ruminants can eructate gases in amounts that far exceed the maximum rates at which they could be produced by fermentation, and concluded that the excessive rates of gas production per se would not cause the clinical signs of bloat. Furthermore, unless the gas bubbles were stabilized as a froth, the bubbles would rise through the rumen contents and be eructated with the gas cap. The eructation (β) contractions are most commonly involved with clearing the cardia of injesta and redistributing it to allow access to the cardia by the gas cap (Reid and Corn-wall, 1959; Reid, 1963; Titchen, 1968; Akester and Titchen, 1969). Rumen fill appears to be an important factor in accounting for the relationship between eructation and ruminal contractions. Dougherty and Meredith (1955) elucidated this system of behavior by insufflating gas into ruminally cannulated sheep and observing a main contraction in the dorsal sac, which caused the large volume of gas above the rumen fluid level to be displaced forward.

At the same time they recorded contractions of the rumino-reticular fold which lifted up and held back the forward surge of solid digesta. Stevens and Sellers (1960) also recorded a lowering of the rumino-reticular digesta level to as much as 10-15 cm and a raising of the posterior dorsal sac of 10 cm in ruminally cannulated sheep that were insufflated to an intraruminal pressure of 30 mm Hg in the dorsal sac. Dougherty (1940) and Stevens and Sellers (1959) also noted that if the rumen was insufflated with gas experimentally, there was an increase in the frequency of the β contractions and in eructation.

In spite of the strong evidence that eructation is associated with β contractions, Clark and Quinn (1945; reported by Hungate, 1966) demonstrated that eructation could occur when the rumen was paralyzed with cyanide.

Receptor Sites

Dougherty et al. (1958) reported that the eructation reflex is stimulated by distension of the rumen and that the receptors capable of inhibiting eructation were located in the vicinity of the cardia. In this study they isolated a small pouch of the rumino-reticulum adjacent to the cardia and demonstrated that gaseous insufflation of this small compartment initiated the eructation reflex. Liquid pressure failed to induce a complete eructation reflex. Although little is known about the mode of control of the cardia, Stevens and Sellers (1959) noted that the opening of the cardia was usually associated with rumen contractions, even when the cardia was kept completely clear. They further observed that the rate of eructation varied with the rate and amplitude of ruminal contractions, even when the cardia

was exposed continuously to gas, and they suggested the presence of a volume-type tension receptor in the cardia.

If the cardia is covered with fluid (including froth) the reflex opening of the cardia does not occur (Dougherty et al., 1958). Presumably, receptors exist at the cardia which can differentiate between gas and fluids.

Leek (1969b) has reported the greatest concentrations of tension receptors in the medial wall of the reticulum, in the rumino-reticular fold, in the lips of the esophageal groove and in the medial wall of the cranial dorsal sac of the rumen.

Weiss (1953) found that if sheep were made to stand on a 30° ramp the frequency of secondary cycles increased if the hind quarters were at the raised end of the ramp. He concluded that gas was accumulating in and distending the caudal region of the dorsal sac, which he attributed as the cause of eructation. This conclusion was supported by Stevens and Sellers (1959), but disputed by Leek (1969a). The latter repeated Weiss' experiment but, with the aid of a cannula, maintained a constant gas pressure within the rumen. The results were the same so Lee concluded that increased fluid pressure in the cranial regions of the rumen was providing the stimulus for increased β contractions. It would seem logical that the receptors involved apparently respond to tension or stretch (Stevens and Sellers, 1959), and that perhaps the β cycles are initiated by tension receptors in the cranial dorsal sac. While a rise in pressure in the region of the cardia would trigger the tension receptors there to facilitate the opening of the cardia to allow the other events of eructation to occur (Dougherty et al., 1958).

The quest for the identification of receptor sites was aptly

summarized by Stevens and Sellers (1959; P. 462) ". . . that the problem

of characterizing rumino-reticular receptors is not one of finding

structures which could act as receptors, but assigning functions to

the structures already described."

Sensory Inputs Which Effect Ruminal Motility

Excitatory Inputs. Iggo (1955, 1956) and Leek (1969a) have

identified low-threshold (about 4 mm Hg) tension receptors located in

the musculature of the medial wall of the reticulum as being the main

excitatory input to the gastric centers. These receptor sites monitor

changes in reflex amplitude, frequency and duration of the mixing con-

tractions (Leek, 1969a). Whenever intraruminal pressure becomes greater

than 4 mm Hg there will be an increase in the mixing cycle contractions.

This threshold is also believed to be related to mild hypermotility

immediately after feeding with a mild degree of bloat (Leek, 1969a).

This mechanism would also account for a depression in motility after

a period of starvation or as a secondary feature to any disease which

results in anorexia; which would both result in hypomotility below

4 mm Hg (Leek, 1969a).

Acid receptors in the abomasum have also been identified by Titchen

(1958) as being another source of excitatory sensory input. Leek

(1969a) regarded this mechanism as being primarily concerned with main-

taining optimum volume of abomasal contents. Hydrogen ion concentra-

tions fluctuate with the content fill in the abomasum. As the abomasum

becomes empty, there is an increase in HCL concentration and a

consequent increase in motility, mostly in primary cycle contractions. As rumen contents begin to flow into the abomasum there is a decrease in abomasal pH, due to dilution of abomasal acid secretions by the rumen contents (Leek, 1969a).

Inhibitory Inputs. These inputs counteract the above excitatory sensory inputs. One of these inputs arises from high threshold tension (about 20 mm Hg) receptors located in the reticulum. These tension receptors are normally activated at the peak of the reticular contraction (Leek, 1969a). This threshold would also account for the more abnormal conditions such as severe bloat and ruminal compaction, the latter being attributed to the feeding of extremely low quality forages. The overall effect of surpassing this high pressure threshold would be ruminal stasis (Leek, 1969a).

Titchen (1958) identified another inhibitory sensory input by observing an inhibition of primary cycle movements due to distension of the abomasum and assumed that these observations were due to the presence of tension receptors in the abomasum. This reflex would appear to act in opposition to the acid receptor mechanism of the abomasum by decreasing the flow rate of rumen contents into the abomasum. This behavior also accounts for the observance of hypomotility which is exhibited in the presence of abomasal impaction and abomasal displacement (Leek, 1969a).

Intraruminal Pressure and the Physiological
Effects of Bloat

Colvin and Daniels (1965) reported that when the rumen is at rest,

the gas pressure is near one atmosphere. A sharp spike in either a primary or secondary contraction will cause the pressure to increase markedly. During severe cases of bloat resting pressures may be as high as 70 mm Hg (Boda et al., 1956; Colvin et al., 1958). Dougherty et al. (1955) have indicated that there is considerable difference between animals and the amount of intraruminal pressure they can tolerate. They observed one sheep that could tolerate an intraruminal pressure up to 100 mm Hg when insufflated with oxygen gas. However, when the same animal was insufflated with carbon dioxide at 60 mm Hg, the animal collapsed within three minutes. These researchers, along with Davis et al. (1965), postulated that the increased absorption of carbon dioxide, obstruction of venous blood return and mechanical interference with respiration were the obvious effects of bloat. Reschly and Dale (1970) reported in experimental studies with goats that rumen insufflation pressures of 40 mm Hg resulted in increased blood pressure and decreased cardiac output which they presumed to be due to increased peripheral resistance caused by the obstruction of slow venous blood return. The net effect of the above events is the animal dies of suffocation.

Histamine and Rumen pH

There is a considerable body of data in the literature regarding the distribution of histamine in the various tissues; which usually varies in concentration from 0-75 μg per gram of wet tissue. This concentration also varies with age and is very low in fetal tissue (Tabor, 1954). Histamine has been considered as a possible factor in contributing to the cause of bloat (Dougherty, 1942). It has been well

established that intravenous injections of histamine into sheep will cause paralysis of the rumen (Dougherty, 1942; Duncan, 1954; Dain et al., 1955) and cessation of eructation (Dain et al., 1955). Hungate (1966) reported the work of Shinozaki (1957) who found no response of the physiological condition of the rumen to orally administered histamine. He attributed this to poor absorption of histamine through the rumen epithelium.

Concern of elevated ruminal histamine concentrations has generally been in association with conditions which lead to acute acidosis and the subsequent decarboxylation of histidine to form histamine. Dain et al. (1955) reported that as a ruminal pH value of 5.0 was approached, histamine formation became evident. Lowering of the pH environment in the rumen, due to the fermentation of large amounts of carbohydrates, resulted in an increased histamine concentration to over 70 μg/ml. Van der Horst (1961) has shown that ruminal fluid concentrations of amino acids and amines can be increased by incubating ruminal fluid with glucose. Sanford (1963) further reported that the increase in histamine content depended on a lowering of the pH, and marked increases in histamine concentration occurred when high acid conditions (pH 4.0 - 4.5) were prolonged. He further suggested that histamine formation might be the result of a change in rumen microflora brought about by an altered rumen environment. This conclusion was based on his observations that incubation of normal ruminal fluid with histidine for periods of 3 to 4 hours with or without the addition of glucose did not result in any histamine formation.

Dain et al. (1955) along with Mangan (1959) reported a ruminal pH threshold for the formation of histamine of 5.0 to 6.3. The

decarboxylase reaction is facilitated by bacteria (Mellanby, 1912) and has been extensively studied (Hanke and Koessler, 1924; Epps, 1945; Gale, 1940, 1945, 1946). Hanke and Koessler (1924) confirmed this threshold for decarboxylase activity and further speculated

> . . . that the production of amines for the amino acids by microorganisms seems to be a protective mechanism and is resorted to when the accumulation of H ions within the organism's protoplasm is incompatible with its normal life processes. The amines can be thought of as reaction buffers (pp. 865-866).

This view point was generally accepted, but Gale (1940) suggested that the formation of decarboxylase activity in an acid environment may be due to the organism's inability to utilize carbohydrate and other substrates at this pH. Gale (1946) offered another possibility that the decarboxylation of the amino acids provided an important source of CO_2 for the organisms, since at an acid pH very little carbon dioxide remains dissolved.

Animal Factors Associated with Frothy Bloat

Individual Susceptibility

While different feeds produce different amounts of gas in the rumen the amounts formed do not affect an animal which is not normally susceptible to bloat. Therefore, the condition of bloat is in part a characteristic of individual animals. The work of Knapp et al (1943) and Hancock (1954) suggested that the susceptibility to bloat is inherited. Furthermore, animals vary in susceptibility to bloat-provocative feeds (Knapp et al., 1943; Barrentine, et al., 1954; Hancock, 1954; Hungate, et al., 1955; Mendel and Boda, 1961; Clarke and Reid, 1970). Clarke and Reid (1970) reported that bloat-limiting factors may not

be the same in animals of different susceptibility when they are not

bloating. These observations focus attention on animal factors and

on changes across time which make the animal susceptible to bloat

during some periods and resistant to bloat at other times. This

characteristic increases the difficulty of defining the underlying

etiologic factors of bloat. Evidence indicating an intrinsic sus-

ceptibility to bloat due to genetic inheritance includes the following:

1) prevalence of bloat in progeny of particular sires (Hancock,

1954; Johns, 1958; Johns et al., 1958; Reid et al., 1972),

2) similarity in the bloating behavior of monozygotic twins

(Hancock, 1954),

3) difference in susceptibility of cattle of different breeds

(Miller and Frederick, 1966; Reid et al., 1972).

Clarke and Reid (1974) postulated that the immediate site of action of

factor(s) that determine susceptibility to bloat would have to be

located within the contents of the rumen. On a large scale, where

several animals in a herd are affected by bloat, it is apparent that

some factor, probably a feed component present in abnormal amounts,

is affecting more than the individual bloat-prone animal and causes

detrimental effects in both sensitive and insensitive members of the

herd. Consequently, under certain conditions a feedstuff will fre-

quently cause bloat in animals eating it, and must be attributable

to plant components.

Saliva

Saliva is produced in copious amounts by five sets of paired glands

and three unpaired glands. The parotid glands account for 40-50%

of the total production (Kay, 1958). The daily secretion in
cattle and sheep has been estimated to be 25 to 190 ℓ and 1 to 24 ℓ,
respectively (Bailey, 1961; Church, 1969). Kay and Phillipson (1959),
Kay (1960,1966) and Schneyer et al. (1972) reported that salivation
is under reflex nervous and hormonal control. Factors which influ-
ence the composition and rate of salivation are the nature of feed
(Ash and Kay, 1959; Emery et al., 1961), stimulation by the digesta
in the gut (Comline and Titchen, 1961), mineral status of the animal
(Blair-West et al., 1965) and osmolality of extracellular fluid (Carr
and Titchen, 1972).

Kay (1960) classified the glands and their secretions into serous,
mucous and mixed. He described serous saliva as thin, high in carbon-
ate, strong buffering capacity, low in protein and little mucoprotein.
Whether the animal is eating, ruminating or resting, serous saliva
is continuously being secreted. Mucous saliva is thicker and contains
more protein and mucin. This type of saliva is secreted predominantly
at the time of feeding.

Various research groups have attempted to discover the role of
saliva in frothy bloat. At present its role is controversial. Weiss
(1953) postulated that bloat was the result of a reduction in saliva
flow, as succulent alfalfa will not provide a strong stimulus for
salivation. Consequently, less saliva was secreted, which increases
the viscosity of the rumen fluid and froth. Meyer et al. (1964) have
confirmed an inverse relationship between salivation and water content
of feed and that cattle produce less saliva when eating succulent
young legumes than when eating mature plants. Phillipson and Reid

(1958) noticed that distension of the rumen during bloat initially stimulated salivation, but that prolonged distension proved inhibitory.

Johns (1958), and Mangan (1959) postulated that an increase in mucoprotein-containing saliva abetted bloat by assisting in the formation of a stable, viscous foam. Nisizawa and Pigden (1960) showed that sialic acid constituted 20 to 30% of the mucoprotein and was responsible for the viscous nature of saliva. The strong negative charge of sialic acid-containing side chains of the protein (Gottschalk, 1960) are attributed to causing the increase in viscosity of saliva.

On the other hand, Fina et al. (1961), Van Horn and Bartley (1961) and Mishra et al. (1968) suggested that the mucoprotein found in saliva serves as a foam-inhibiting and foam-breaking agent and postulated that mucinolytic bacteria have a role in breaking down the anti-foaming mucins in the saliva. However, Mendel and Boda (1961) could not demonstrate differences in the mucin content of saliva from animals with high or low susceptibility to bloat. They found higher bicarbonate concentrations in saliva of bloat susceptible cattle.

In conclusion a large salivary secretion could decrease bloat by buffering a fall in pH. But it could also increase the severity of bloat by increasing the production of carbon dioxide and subsequent froth formation. Studies on acid-released carbon dioxide have been made by Johns (1958), Mangan (1959) and Gupta et al. (1962). They estimate the daily production of carbon dioxide from bicarbonate in the saliva during volatile fatty acid production to be greater than 250 ℓ. As mentioned earlier, the animal should be able to handle this volume of gas; however, this amount of carbon dioxide would be more

critical if it occurred concomitantly with the factors that contribute to maximum foam strength and stability.

Saliva clearly has the potential of being an important factor relating to bloat, but much more data are needed to reach conclusions concerning its specific role.

Plant Factors Associated with Bloat

Soluble Proteins, Nitrogen and Nucleic Acids

Plant proteins are significant factors in the formation of stable foams (Miltimore et al., 1970). The literature indicates that the protein foaming agents are very sensitive to pH and temperature (Pressey et al., 1963b; Buckingham, 1970). These researchers also reported the presence of foam inhibitors in alfalfa proteins and suggested that the plant contains a complex system of foaming agents and inhibitors, and that bloat potential is a delicate balance of these different parameters. Bartley and Bassette (1961) support the major role of protein in foam formation, but Head (1959) postulated that the entrappment of copious gas into small bubbles in the rumen liquor was attributed to the presence of pectin and/or hemicellulose.

Singer et al. (1952) reported that soluble leaf proteins may be divided into two main groups on the basis of molecular size. Fraction I protein is homogenous and has a molecular weight of about 555,000. Trown (1955) has identified this protein to be enzymatically active as ribulose-1,5-diphosphate carboxylase. It makes up most of the protein of chloroplasts of green leaves (Lyttleton and Ts'O, 1958).

Weissbach (1956) described its role as being associated with the fixation of carbon dioxide during photosynthesis and therefore is a major component of most green plants. Fraction II protein is made up of all the other soluble leaf proteins not included in Fraction I. Fraction II mixture of proteins has a molecular weight, from 10,000 to 20,000.

Fraction I protein is commonly referred to as the foam-stabilizing protein, although Jones and Lyttleton (1973) along with Howarth et al. (1973) reported that both fractions contribute to the foaming properties of legumes. Stifel et al. (1968a) have shown that soluble leaf chloroplasts and total protein were directly related to the severity of bloat in cattle and sheep. Howarth et al. (1975) reported the minimum soluble protein N concentration at which bloat occurred was 0.214% of dry weight. These workers also found correlation coefficients between bloat incidence and total nitrogen (N), insoluble N, soluble non-protein N and soluble protein N to be $r = 0.25$ ($P < .05$), $r = .18$ ($P < .1$), $r = .10$ ($P > .1$), and $r = .34$ ($P < .005$), respectively. Miltimore et al. (1964) reported that nitrogen content of alfalfa was correlated ($r = .54$) with the degree of bloat. Miltimore et al. (1970) reported that 1.36% Fraction I chloroplast protein would cause bloat. McArthur and Miltimore (1969) suggested that the threshold concentration for bloat was approximately 2% for Fraction I protein. They also mentioned that bloat provocative forages were higher in soluble protein than non-bloating forages and that of the soluble protein, almost one-third was Fraction I, compared with one-sixth or less from non-bloating forages. In the same study these researchers also found that there were no differences in RNA or DNA content. They concluded that although soluble protein might be used as an indicator to estimate bloat

potential, the Fraction I protein differences between bloat provocative and non-bloat forages were proportionately much larger and therefore would be more accurate for estimating the bloat potential of forages.

McArthur et al. (1964) proposed a hypothesis for the role of Fraction I protein in foam formation. In view of the fact that Reid (1959) considered the release of plant cell contents upon chewing to be a factor in bloat, McArthur et al. (1964) postulated that with the release of chloroplasts into the rumen, the Fraction I protein was readily soluble in the rumen fluid in a spherical shape. If the molecule reached the surface of the rumen fluid without undergoing microbial degradation, it would uncoil and become insoluble. These researchers related the stability of foam to the relative amount of this surface denatured protein present in the rumen fluid. However, the cohesive forces between the molecules will promote coagulation. Consequently, protein is not surface active and will not stabilize a foam. Upon agitation, protein coagulation was enhanced due to the strong forces between the molecules and there was a consequent reduction in foam stability. It would then appear that the significant factors involved in the foaming agent concentrations are: the rupture of the leaf cells, proteolytic activity in the rumen liquor and agitation of rumen contents. Jones and Lyttleton (1969) lent support to these observations concerning protein denaturation by using gel filtration analysis similar to that reported by McArthur et al. (1964) in obtaining similar results.

Another event that has been reported to be critical to events leading to surface-denaturation of proteins is the pH of the rumen. Both fractions of chloroplast proteins have been reported by Jones and

Lyttleton (1972a) to produce strong foams within the pH range of 4.4
to 6.0. Laby (1969) and McArthur and Miltimore (1969) report that the
soluble leaf proteins of red and white clovers produce foams of maxi-
mum strength within a pH range of 5.4 to 6.0. Jones and Lyttleton
(1972a) concluded that both protein fractions have maximum foam strength
capabilities within this pH range, with Fraction I having a sharper
maximum at about pH 5.8.

Soluble Carbohydrates

Quinn (1943) considered that an easily utilizable carbohydrate
present in high concentration was in a large part responsible for bloat-
ing, and showed that the incubation of rumen liquor with glucose solu-
tion rapidly increased gas formation. Head (1959) concluded the same
and postulated that plant pectin and hemicellulose were the substances
responsible for the foam formation in the rumen of bloated cattle. He
further stated that higher gas production must be in concert with the
surface-active agents in order for bloat to occur, and that one without
the other would have no detrimental effect on an animal.

In reviewing the literature, most of the findings involving carbo-
hydrates and bloat indicate that pectic substances are most commonly
involved. Wright (1961) and Dehority et al. (1962) reported that pectin
does not remain in the rumen long but is degraded rapidly by extracellu-
lar enzymes and microorganisms. Conrad et al. (1958, 1961) has demon-
strated that pectic substances may comprise as much as 8% of the
dry matter in some grasses; this represents a major source of carbohy-
drates injested by ruminants. Pressey et al. (1963a) reported that
pectic substances make up 14 to 15% of the dry weight of legumes,

but found no correlation with either water soluble or total pectin. However, Pressey et al. (1963b) in another study found that added pectin increased foam stability of alfalfa extracts in vitro. Wright (1960, 1961a) observed pectic gel formation and entrappment of gas along with an increase in viscosity of rumen digesta in vitro and postulated that the action of pectin methyl-esterase (Gupta and Nichols, 1962) in vivo assisted in this gel formation. Nichols and Deese (1966) and Nichols et al. (1968) reported a significant association (P < .01) between the incidence of bloat and the intake of this enzyme by the animal. Penn et al. (1966) suggested that the presence of a natural occurring inhibitor of pectin methyl-esterase is a contributing factor in preventing bloat in non-bloating animals.

Wright (1961a) and Hungate (1966) concluded that carbohydrates are undoubtedly involved with gas production, but that their effect on foam stability is doubtful except when they stimulate rumen microbes to produce slime.

Saponins

Saponins have a controversial role in frothy bloat. The capacity of saponins to stabilize foams and the abundance of saponins in legumes is well known, and has stimulated much interest in finding a possible role of saponins in bloat (Lindahl et al., 1954).

Froth due to saponins is stable at pH 5.0 but negligible above 6.0. The latter would be considered to be lower than the pH of rumen digesta in legume-bloated animals (Mangan, 1959). Following the foam studies of Mangan (1959), saponins have not been considered to be

major agents in the genesis of bloat froth. Mucogenic strains of

Butyrivibrio which attack saponins have been found in the rumen of

steers fed fresh alfalfa (Gutierrez, et al., 1958; Gutierrez, et al.,

1959; Gutierrez and Davis, 1962).

Tannins

Tannins are polyphenolic compounds that react with proteins to

form a leather-like substance that is insoluble and has reduced di-

gestibility.

Miltimore et al. (1970) reported a positive association ($P < .05$)

between Fraction I protein and tannins. However, Cheek (1971) pro-

posed that tannin-saponin complexes may, in part, explain the low bloat

potential of high tannin legumes. Kendall (1964) also showed that

foam production in vitro was inhibited by tannins. When he added poly-

vinyl pyrrolidone (PVP), an agent known to complex with tannins, the

ability of rumen fluid to foam was restored. This finding led Kendall

(1966) to postulate that foam production in the rumen might be inhibited

by plant tannin. Although Clarke and Reid (1974) suggested no apparent

association between tannins and bloat, these researchers cited the

work of Hutton and Coote (1966) who examined nineteen species of legumes

and found no correlation between tannin content of leaves and the ab-

sence of bloat.

Minerals

Cooper (1957) reported that a high-calcium, low-phosphorous

ratio and a high-nitrogen, low-phosphorous ratio cause bloat. Many

salts are foam stabilizing agents. Addition of as little as 0.63 M

NaCl increased the foaming capcity of cytoplasmic proteins and maximized foam stability (Mangan, 1959). On a molar basis Ca^{+2} was twice as efficient as Na^{+1} in enhancing foam stability.

The literature suggests that minerals can be implicated in many different manners to promote bloat. Wright (1961a) suggested that pectate formation might reduce the available amount of calcium for stabilizing foams. Stifel et al. (1968a) found strong correlations ($r = .85$ to $r = .92$) between bloat and the extent and strength of calcium and magnesium binding to Fraction I chloroplast protein at pH 5.5. These investigators suggested that any factor(s) which promote protein denaturation would enhance bloat. They concluded that the mineral binding effect would salt out Fraction I protein and therefore stabilize rumen foam. The relationship of Ca^{+2} and Mg^{+2} binding to bloat may have more meaning in terms of the proposal by McArthur et al. (1964). They suggested that Fraction I protein acts as a ruminal foaming agent only in a denatured state. Factors such as polar lipids would actively compete with protein for mineral and metal ions which would decrease surface denaturation of the protein and thus act as antifoaming agents. This would agree with Ross and Haak's (1958) foam inhibition theory where they observed that anti-foaming agents act by absorbing foaming agents from the surface of intralamellar bulk solutions.

Trace Metals

Miltimore et al. (1970) have shown that the contents of nickel and zinc in alfalfa were associated ($P < .05$) with bloat. Harris and

Sebba (1965) identified protein as the foaming agent which binds Ni^{+2} on its negatively charged sites, which could well be a natural occurring example of ion flotation, where the protein acts as the collector and the metal ion is collected. In each case a decrease in stability of the foam was accompanied by a decrease in the nickel concentration of the foam. They further noted that the nickel concentration decreased with time. They also observed that in fresh alfalfa the nickel appeared to be available for attachment to the protein, while in aged alfalfa, although the nickel was still present, it was no longer available. When they added traces of nickel sulfate to the aged solutions, foam stability immediately increased substantially. The presence of nickel was only detectable when concentrated in the foam. Other possible changes over time might involve changes in protein conformation and configuration. Their data also showed strong evidence of metal binding to various components of Fraction II protein.

Lipids

Mangan (1959) noted that the stability of froth was less in the presence of alfalfa chloroplasts. Removal of the chloroplasts increased the stability of rumen foam in vitro. When the lipids were removed from the chloroplasts, the chloroplasts were no longer effective in disrupting the froth.

Chloroplasts contain lipids and galactosyl and glycerol esters of linolenic acid (Weenink, 1962; Benson et al., 1959). Both bacteria (Wright, 1961B) and protozoa (Wright, 1959) of the rumen have been identified as being able to hydrogenate lipid. As lipid is hydrogenated, the tendency of the lipid to break up froth increases. Also Oxford

(1959) showed that ciliate Epidinium could aid foam formation by removing chloroplasts from the rumen at a rapid rate. The action of penicillin in preventing bloat is thought to be due, in part, to the inhibition of bacteria that modify lipid (Mangan et al., 1959; Wright, 1961).

Stifel et al. (1968b) proposed a competitive relationship between the protein in alfalfa foam and lipid. These authors suggested that polar lipids compete for mineral and metal ions which bind to Fraction I protein and decrease protein denaturation. They further postulated that with maturity, the saturation of fatty acid content of phospholipid of plants increased (Klopenstein and Shigley, 1967)., and that calcium binding also increased. Thus, the incidence of bloat would be reduced. The inverse is thought to be true with young growing plants. The phospholipid content would be high in unsaturated fatty acids, thus more calcium would be available to bind to protein to increase denaturation, so viscosity and the incidence of bloat increase.

This theory has a lot of merit, but paints only a portion of the complete picture. Stifel et al. (1968a) found a strong negative correlation ($r = -.67$) between total leaf lipids of alfalfa and bloat; while Miltimore and fellow workers (1970), although finding lipid concentration and bloat not to be correlated statistically, reported that as the Fraction I protein increased from 2.7 to 3.8%, the average lipid concentration also increased from 4.0 to 6.5%.

Characteristics of Wheat Forage

Green winter wheat pasture is an excellent high quality forage. Johnson (1973) and Johnson et al. (1974) have demonstrated that the

wheat plant is high in protein and low in fiber. Although no digestibility studies were run, these workers suggested that wheat forage was highly digestible, since only 3% lignin was present in the herbage. Horn et al. (1974) reported that in vitro dry matter disappearance of winter wheat pasture ranged from 48 to 76%. Monson (1978) attributed the high digestibility of wheat to its unique mode of being digested by bacteria, not only from the particle ends, but also from the sides.

Wheat pasture during the grazing season will usually vary from 20 to 30% crude protein content. Johnson et al. (1973) stated that up to 27% of the total nitrogen was non-protein nitrogen.

Wheat pasture contains between 10 and 30% soluble carbohydrate on a dry matter basis (Johnson, 1973, 1974). One might conclude that the soluble carbohydrate content of wheat forage contributes to the rapid evolution of gas during the extensive fermentation of these highly digestible substrates by the rumen microorganisms. Yet wheat forage is extremely low in soluble carbohydrates during the fall and spring, which is usually the time of the grazing season when the incidence of bloat is greatest (Wilson, 1975). Horn et al. (1974) also reported that for most of the grazing season the soluble carbohydrates in wheat forage were low, relative to the amount of protein present.

The mineral content of wheat pasture has received some attention in consideration to the incidence of bloat. Horn et al. (1974) suggested that wheat pasture may provide a diet too low in available calcium and magnesium and too high in phosphorus; which would place wheat pasture opposite to the calcium:phosphorus ratio of alfalfa.

Clay (1973) observed potassium concentrations of 2 to 3% of
the forage dry matter. High potassium concentrations may decrease
membrane excitability of the ruminal musculature and decrease ruminal
motility (Parthasarathy and Phillipson, 1953).

Horn et al. (1976) collected samples from pastures where bloat had
or was occurring. They found that wheat forage samples taken from pas-
tures where bloat was exhibited contained less (P < .05) dry matter and
neutral-detergent fiber. Total soluble nitrogen, soluble protein
nitrogen and soluble non-protein nitrogen were all higher (P < .05)
from bloat provacative pastures.

Microbial Populations Associated with Bloat

Investigations concerning the role of rumen microbes in the
etiology of bloat have been quite extensive. Jacobson et al. (1958)
noted that the changes in the bacterial populations of bloating ani-
mals were also marked by changes in the ratios of volatile fatty acids
found in the rumen. Gutierrez et al. (1959) also demonstrated a
change in the metabolic behavior of rumen flora with the onset of
bloat. However, the majority of more recent investigators have not
been able to demonstrate any differences in fermentation rates between
bloating and non-bloating animals (Gutierrez and Davis, 1962; Clarke
and Hungate, 1971). In addition Bryant and colleagues (1960), along
with Clarke (1964) and Clarke and Hungate (1971) have all concluded
that differences in numbers of species of ruminal microorganisms are
not related to the occurrence of bloat.

Gutierrez et al. (1958) indicated an association between poly-
saccharide slime formation and the possible role of saponins in

causing bloat. Hungate et al. (1955) also postulated that the high

content of readily available carbohydrate in lush legumes might provide

sufficient substrate to support a copious production of slime. Vari-

ous workers also have reported that the slime for feedlot bloat con-

tained nucleic acid as DNA (Gutierrez et al., 1961), whereas the slime

from legume forages contained only RNA (Gutierrez et al., 1963).

Gutierrez and Davis (1962) and Gutierrez et al. (1963) thought that the

slime from alfalfa bloat came from the plant and was the by-product

of microbial utilization of the carbohydrate moiety of the saponin

molecule.

Clark (1965) suggested that the cell contents of holotrich

ciliates may enter into the rumen fluid via rupture of the cell walls

of the ciliates due to excessive storage of the abundant amounts of

soluble carbohydrates found in succulent legumes. Jones and Lyttleton

(1972b) lent support to this hypothesis by demonstrating that the

particle free cell contents of rumen ciliates do produce rigid foams

within a pH range of 5.5 to 6.5. This hypothesis is attractive since

the spillage of the cell contents along with nucleic acids would

greatly increase rumen fluid viscosity (Hungate, 1966). Yet Bryant

et al. (1960) found no differences in slime production between bloat

susceptible and non-susceptible animals.

<div align="center">The Chemical and Physical Properties of Froth</div>

Protein Monolayers

Only a brief discussion will be presented here. For a more

detailed discussion, the reader is referred to an excellent review

article by Kitchener and Cooper (1959).

Foam is a two phase system, comprised of a discontinuous phase, a gas, and a continuous phase, an aqueous solution. Monolayers of surface-active molecules located at the liquid-gas interface maintain the foam. Upon breaking-up, foams exhibit no scum. A froth, likewise, is a two phase system, consisting of a discontinuous gas phase and a continuous liquid phase. Froths are unique foams in that they are stabilized by insoluble monolayers at the liquid-gas interface. These monolayers are not visible. But when the froth collapses, a scum becomes visually evident due to the agglomeration of the froth molecules at the interface (Kitchener, 1964).

The stabilizing monolayer is very fluid and elastic in nature. Protein monolayers can fulfill these attributes and are recognized as being excellent frothing agents. Since froth bubbles are continually being bombarded with small mechanical stresses, the elastic properties of froths appear to be very important to the stability and persistence of a froth. These stresses need to be relieved in some manner, or the bubbles would collapse (Labby, 1975).

Froth Persistence and Stability

Based on the observation that a pure liquid will not froth, there remains little doubt concerning the necessity of a froth stabilizer at the interface. Bubbles formed on the interior of a solution of pure water and allowed to rise to the surface stop for a short time interval, often only a fraction of a second, before bursting into the air. This time interval is a measure for the persistence of the film (Burcik,

1950). The presence of any monolayer on the surface will allow the bubble to persist for a longer time, depending on the concentration of the surfactants at the liquid-gas interface. Several bubbles at the surface of a surfactant solution behave by flowing towards one another and forming clusters. Excess surface pressure appears to push the bubbles together, based on the observation that the bubbles push towards the sides of the vessel and collapse when very little surfactant is present (Kitchener, 1964). The behavior of bubbles is discussed in more detail in A. M. Gaudin's (1957) text, Flotation.

The boundaries of bubble clusters are curved since froth bubbles all have different diameters and thus are not characterized by plane boundaries between adjoining bubbles. Froths are fundamentally unstable systems and this difference is greatly affected by the variation in bubble size. The thermodynamic instability of froths is also attributed to the draining and bursting of froth films. Since the pressure of the gas is inversely proportional to the bubble radius, gas diffuses slowly from the smaller bubbles into the larger ones (Labby, 1975).

A stable froth exists when the film of fluid containing the monolayers does not thin appreciably with time. The fluid flows downward because of gravitation. Brady and Ross (1944) have confirmed that the rate of flow is inversely proportional to the viscosity of the liquid. With draining of the fluid containing the protein monolayers, there is a thinning of the bubble walls until eventually they become so thin that the bubbles break. If the viscosity is increased, the rate of drainage is reduced and the film of fluid surrounding the bubbles will persist for a longer period of time (Labby, 1975). Bikerman (1953) in his book, Foams, defined the stability (persistence or life span)

of a froth as being determined by the rate of bursting of films and not by the rate of drainage. Labby (1975) reported that upon the initiation of bubble collapse between bubble septa, the gases from the individual bubbles merge, producing new bubbles, whose volumes were the sums of the previous ones, with slightly less pressure. The shock of this rupture is transmitted to other bubbles with consequent further collapse.

As a froth ages it is made up of larger bubbles and thinner septa. It has been observed by the author that the froth bed of rumen fluid consisted of large bubbles on top, which would contain less liquid surrounding the bubbles, and a lower level of smaller bubbles which would be more wet. By administering a constant stream of gas, one observes a continual evolution of bubbles from the rumen fluid; with the younger bubbles lifting up the older ones.

Kitchener and Cooper (1959) have mentioned other factors that can influence foam stability. Among these are concentration of solute, temperature and the effect of mixing more than one surfactant. The stability of froths is also very sensitive to changes in pH (Mangan, 1959; Wright, 1959).

Environmental Factors Associated with Bloat

A wide range of environmental factors is associated with the occurrence of bloat. Factors that have been considered most important include high humidity, rain, wind, frost, dew, and even drought (Cole, et al., 1945; Johns, 1956). Clay (1973) working with winter wheat pasture noted that during the early fall and spring when the plant

growth is most rapid, the incidence of death loss due to bloat coincided with the above factors. This researcher also observed fewer deaths associated with the spring growth of 1972 (during drought) than during the more normal spring of 1973.

Bailey (1958) noted that bloat was most common with cool nights (below 10 C) and mild days. These conditions, which are often associated with cold fronts, are conducive to the production of high levels of soluble sugar and starch in legumes. This may be a triggering mechanism for bloat (Bailey, 1958).

Clay (1973) suggested that the largest number of deaths due to bloat in wheat pasture stockers was during periods when the weather was fluctuating rapidly and when the forage was succulent and growing rapidly. Horn et al. (1974) examined the grazing behavior of stocker cattle on wheat pasture and observed that wheat pasture stockers stop eating prior to the movement of weather fronts through an area and then consume large amounts of forage after the weather fronts have passed. An increased forage intake might alter the rumen system sufficiently to induce bloat. About 25% of the calves exhibited ruminal distension and were believed to be bloated. According to Hancock (1954) the rate of eating is not important, although Mendel and Boda (1961) reported a higher dry matter content in the ruminal injesta from bloat susceptible cows.

Horn et al. (1976) suggested a subtle relationship between climatic (growing) conditions, soil fertility management and stocking rates as they affect the maturity of forage growth, accumulation of forage and the incidence of bloat.

Various studies have been conducted in Oklahoma to investigate the effect of fertility treatments on the suspected chemical components of wheat pasture believed to be associated with bloat. Johnson et al. (1974) measured wheat sample fractions for the effect of 9 kg N/ha (control) 74 kg N/ha (high-N), 92 kg K/ha (high-K) and high-N + high-K fertilizers on soluble carbohydrate content and total nitrogen. They found no effect of the fertility treatments on the soluble carbohydrate content or the total nitrogen content in plant tissue.

Baker and Tucker (1971) found that the nitrate content of wheat forage increased with increasing rates of nitrogen. However, growing conditions can have a significant effect on nitrate content (Wilson, 1975). Wilson (1975) further suggested that high levels of nitrate in young wheat forage was associated with cloudy weather and light rain. He reasoned that these weather patterns may have allowed nitrates to accumulate in the plant due to reduced nitrate reductase activity. Since moisture must be present for nitrogen to enter the plant root zone and thus become available to the plant (Thompson, 1968), the absorption of nitrogen into the wheat plant during a dry period or season would be considerably reduced.

The research to date appears divergent in explaining the effects of the environment on wheat pasture bloat.

CHAPTER III

RUMINAL MOTILITY OF STOCKER CATTLE GRAZED

ON WINTER WHEAT PASTURE

Summary

Aplitude (mm Hg) and frequency (sec) of ruminal contractions were measured as an index of reduced secondary ruminal contractions being a predisposing factor in the bloating of stocker cattle on wheat pasture. Measurements were obtained at approximately weekly intervals during the 1975-76 and 1976-77 wheat pasture grazing seasons. Ruminal motility was recorded during the 1975-76 grazing season by pressure transducers surgically implanted in the dorsal ruminal sacs of three Herford steers, or by water-filled, balloon cannulae inserted in the dorsal ruminal sacs of four additional Hereford steers equipped with permanent ruminal cannulae, and attached to an external pressure transducer. Data conconcerning ruminal motility patterns during the 1976-77 grazing season was obtained from four Hereford x Angus stocker calves equipped with permanent cannulae situated in the dorsal ruminal sac of each animal. Baseline data was obtained for both years by measuring amplitude and frequency of ruminal contractions prior to placing the steers on pasture and/or after the steers had been removed from the trial pasture. Mean amplitude (mm Hg) and frequency (sec) ranges of ruminal contractions are listed for 1975-76 and 1976-77. Method of motility

measurement is enclosed in parentheses, mean pre- and/or post-wheat pasture control amplitude and frequency values are underscored and the range of data for amplitude and frequency are listed separately. 1975-76 (pressure transducers surgically implanted in the dorsal ruminal sac) 6.7, 33.5; 16.7-24.9,26.5-35.4; 1975-76 (water-filled balloon, cannulae attached to an external pressure transducer) 12.9; 32.2; 17.9-25.2, 28.3-41.4; 1976-77 (implantable pressure transducers placed in the dorsal ruminal sac through small ruminal cannulae) 17.1, 23.8; 11.0-33.5, 15.8-38.0, respectively. Amplitudes and frequencies of ruminal contractions for the two year study, in general, remained fairly constant or increased (P < .05). These data do not indicate that reduced ruminal motility is a predisposing factor in the bloating of stockers grazed on wheat forage.

Introduction

Frothy bloat is a major cause of deaths (2-3%) in stocker cattle grazed on winter wheat pasture in Oklahoma. Eructation of ruminal fermentation gases occurs as a sequel to the secondary ruminal contractions (Sellers and Stevens, 1966). It has also been shown that the opening of the cardia is usually associated with the secondary contractions (Stevens and Sellers, 1959) and that the covering of the cardia with fluids, such as mineral oil, water or rumen ingesta, results in the failure of the cardia to open (Dougherty et al., 1958). The rate of eructation has also been demonstrated to be related to the frequency and amplitude of the ruminal contractions (Stevens and Sellers, 1959). Williams (1955) observed that the primary reticulo-ruminal contractions

occurred at regular intervals during frothy bloat, but that the secondary contractions were absent.

At the present time, there is no experimental evidence to suggest that a reduction in secondary ruminal contractions is a contributing factor to the bloating of stockers on wheat pasture. The objective of this study was to determine if a reduction in ruminal motility occurred as a predisposing factor in the bloating of stocker cattle grazed on winter wheat pasture.

Experimental Procedures

Wheat Pasture

The studies were conducted on twenty hectares of wheat pasture at the Oklahoma State University Dairy Cattle Center. Nineteen kg of Triumph 64 wheat seed per hectare were sown on September 8, 1975, and September 9, 1976. Urea (26 kg/ha) was applied immediately before drilling, and 9 kg/ha of 18-46-0 fertilizer was applied with the seed. Total nitrogen applied per hectare at planting was approximately 14 kg/ha. No additional nitrogen was applied during the remainder of the grazing season.

1975-76 Wheat Pasture Grazing Season

Ruminal motility of three Hereford steers (250 $\pm$ 14 kg) placed on wheat pasture on December 31, 1975, was measured at approximately weekly intervals from January 6, 1976, to February 14, 1976, by means of

pressure transducers[1] (Figure 1) surgically implanted in the dorsal
ruminal sac. A model DMP-4A physiograph recorder[2] equipped with a type
7172 strain gauge coupler and a type 7070 channel amplifier was used
to record the ruminal contractions. The hard wire and electrical leads
connector of each pressure connector was exteriorized and passed
subcutaneously to the region of the lumbar vertebra. Each electrical
leads connector was covered with a water-proof cap during those periods
in which the cattle were grazing wheat forage, and measurements of
ruminal motility were not being made. The amplitude and frequency
of ruminal contractions were evaluated by moving the steers off wheat
pasture, placing them in individual stalls, and connecting the electri-
cal leads to the physiograph recorder. In order to establish baseline
data, amplitude and frequency of ruminal contractions were measured
for three consecutive weeks prior to putting the steers on wheat pas-
ture. While off wheat pasture the steers were fed a ration that con-
sisted of 54% ground alfalfa hay, 32% corn, 7% cottonseed hulls, 5%
soybean meal and 2% minerals and vitamins.

During the 1975-76 grazing period, measurements of steers with the
surgically implanted pressure transducers were interrupted by the loss
of patency of the transducers as a result of the animals scratching
their backs on fence wire and low-hanging tree limbs. The average
number of days for the three steers in which surgical transducers were
used was eighty-one. Therefore, for the remainder of the wheat pasture

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[1]Model No. P6.5; Konigsberg Instruments, Inc.; 2000 East Foothill
Boulevard; Pasadena, California 91107.

[2]Narco Bio-Systems, Inc.; P. O. Box 12511; 7651 Airport Boulevard;
Houston, Texas 77017.
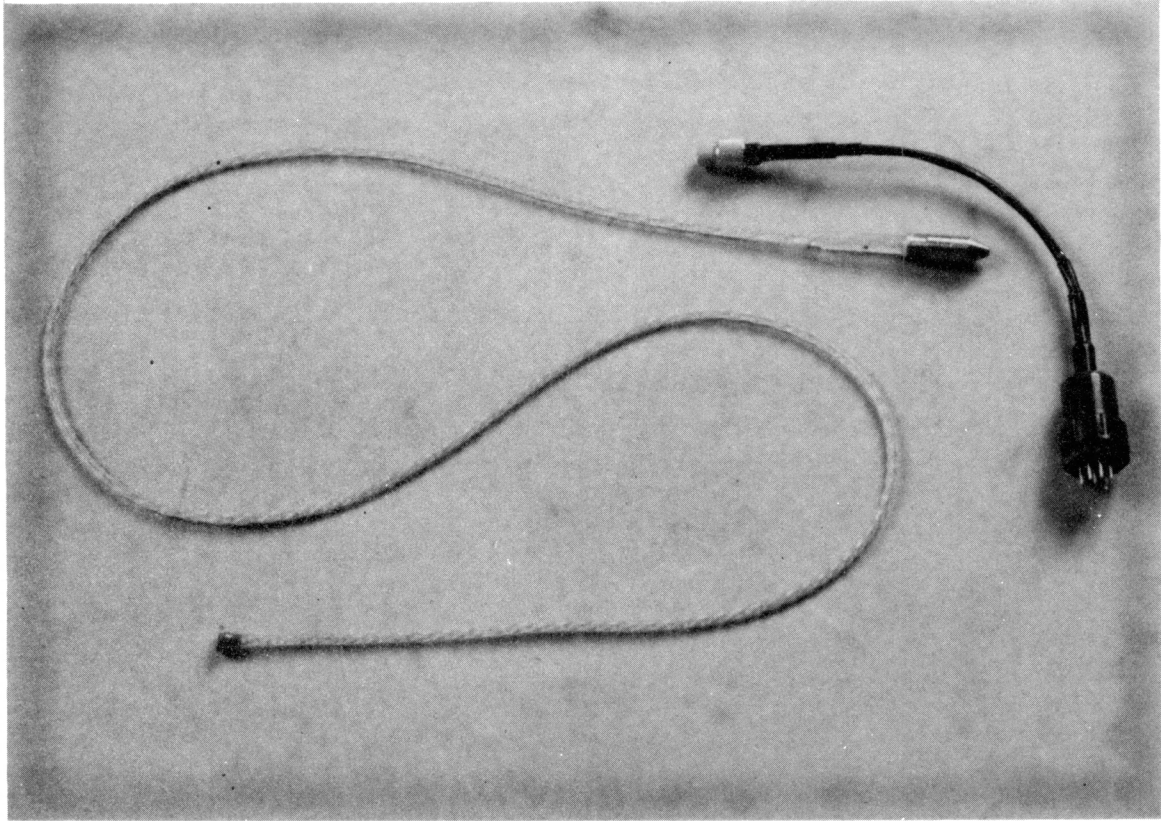
Figure 1. Implantable Pressure Transducer

grazing period, water-filled, balloon cannulae were used. These were inserted into the dorsal ruminal sac through small ruminal cannulae (2.54 cm. I.D.) of four Hereford steers (216 ± 7.0 kg) placed on the pasture on November 10, 1975. The water-filled, balloon cannulae consisted of approximately 210 cm of intramedic polyethylene tubing[3] attached to a piece of stainless steel tubing (12 cm in length); this was passed through a No. 6 rubber stopper, terminating with a No. 0 rubber stopper, to which a balloon was fastened with a copper wire. The water-filled, balloon cannulae were attached to a type P-1000A external pressure transducer (Figure 2). The calibration was established by placing the transducer inside an enclosed glass container (Figure 3). The steers were moved to individual stalls before ruminal motility was measured. Baseline data for these measurements were obtained for two consecutive weeks after the steers had been taken from the wheat pasture. The same control ration was fed as indicated previously.

The average daily weight gains of both sets of cattle on the wheat pasture (143 head days) was (0.93 ± .04 kg/head/day. This reflects the large amount of wheat forage that was available to them.

1976-77 Wheat Pasture Grazing Season

Four ruminally cannulated Hereford x Angus steer calves (243 ± 6.0 kg) were placed on winter wheat pasture on November 13, 1976, and remained on pasture until March 24, 1977. Measurements of ruminal

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[3]PE 240 (1.67 mm I.D., 242 mm O.D.); Clay Adams; Parsippany, New Jersey 07054.
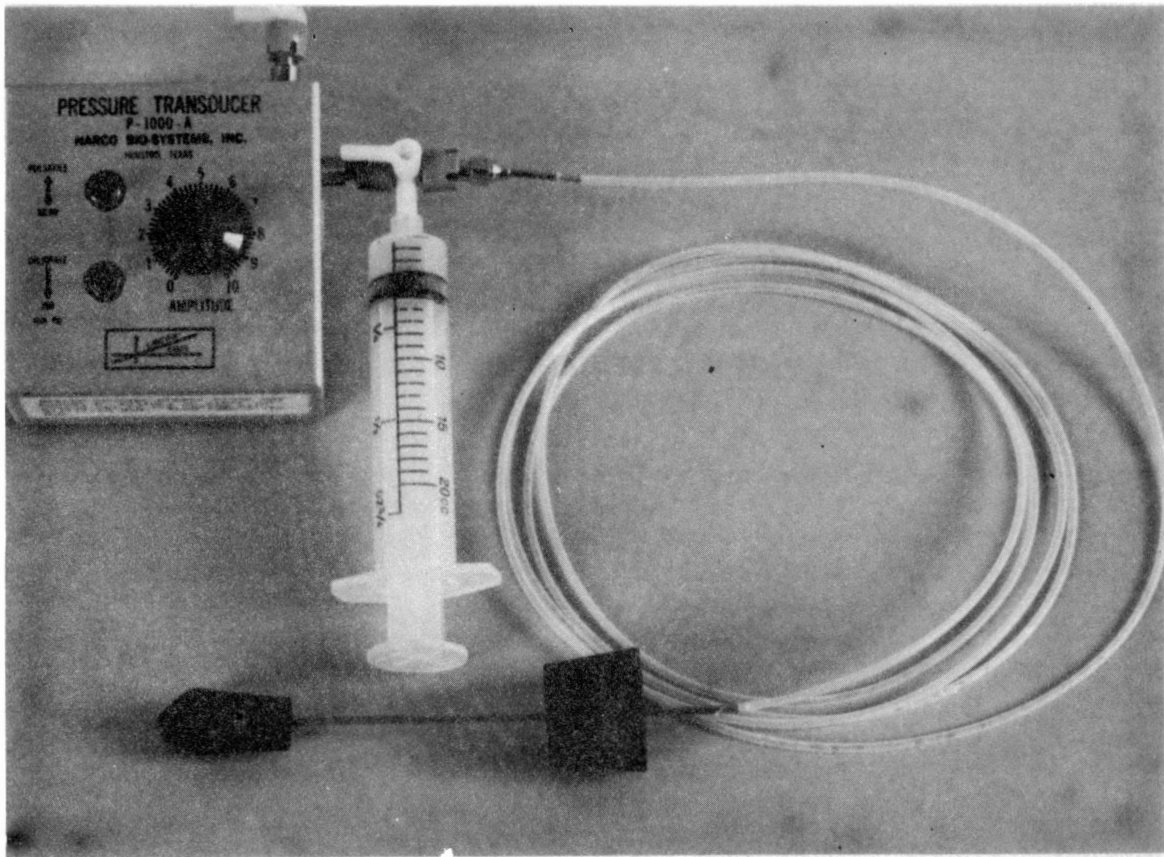
Figure 2. External Pressure Transducer and Water-Filled,
Balloon Cannulae
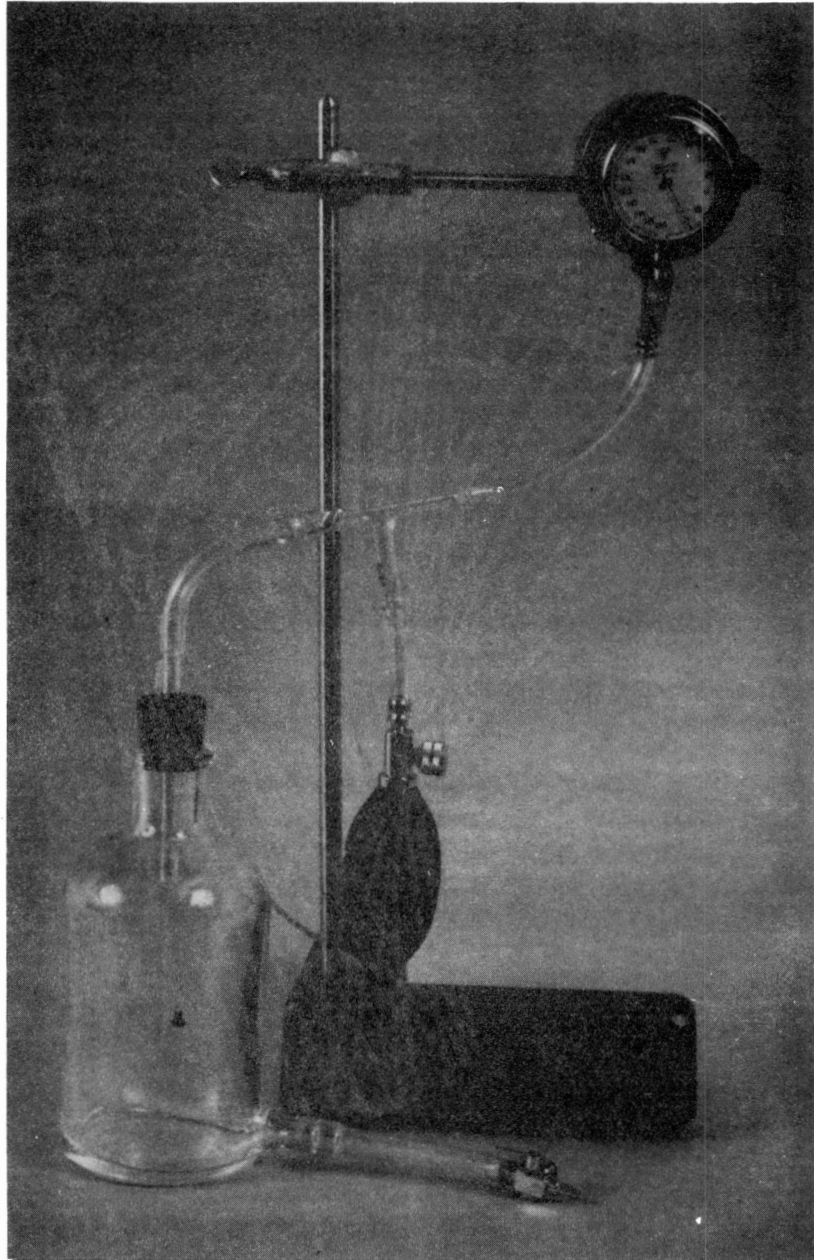
Figure 3.   Enclosed Glass Container and
            Pressure Gauge for Calibra-
            tion

motility were taken from November 23, 1976, to March 15, 1977. The average daily gain of the steers, based on 153 head days on pasture, was 0.95 ± .05 kg/head/ day. Ruminal motility of the steers was measured by placing implantable pressure transducers[1] in the dorsal ruminal sac through small ruminal cannulae (2.54 cm I.D.). The ruminal contractions were measured by moving the steers off wheat pasture, placing them in individual stalls, connecting the transducer to the physiograph recorder, calibrating the recorder by placing the transducer inside an enclosed glass container (Figure 3) and then inserting the transducer into the dorsal ruminal sac, through the ruminal cannula of each animal. The baseline data was obtained by measuring amplitude and frequency of ruminal contractions for two consecutive weeks prior to putting the steers on pasture, and for two consecutive weeks after taking them from the wheat forage. The same control ration as used the previous year was fed.

In both years, no attempt was made to differentiate between primary contractions of the reticulum or to distinguish dorsal secondary contractions from ventral secondary contractions.

Statistical Analysis

The ruminal motility data were analyzed by analysis of variance procedures for a randomized complete-block design with steers as blocks and time on wheat pasture as treatment. Tests of significance between the amplitudes and frequencies of ruminal contractions while on wheat pasture versus the mean pre- and/or post-wheat pasture amplitudes and frequencies were made by use of an LSD protected by a preliminary F test (steel and Torrie, 1960). The date x steer mean square was used as the error mean square.

Analyses of the pre- and post-wheat pasture mean amplitudes and frequencies for the 1976-77 grazing period were compared with an LSD as the test criterion.

Results and Discussion

1975-76 Wheat Pasture Grazing Season

The mean amplitudes and frequencies of ruminal contractions of steers while on wheat pasture and during the pre- and post-wheat pasture periods are shown in Tables I and II. Most of the amplitudes of ruminal contractions were significantly increased (P < .05) when compared to the respective pre- or post-wheat pasture amplitude means. Extremely large amplitudes of ruminal contractions, in the range of 40 to 50 mm Hg, were frequently observed during the grazing period. The frequency of the ruminal contractions was slightly increased (P < .05), when compared to the control mean on March 17, 1976. Significant (P < .05) reductions in ruminal amplitude and/or frequency patterns, were not evident from the data collected.

Since histamine is a potent inhibitor of ruminal motility and eructation, (Dougherty, 1942; Dain et al., 1955), and since the level of dietary protein affects ruminal histamine concentrations (Long et al., 1970), aliquots of rumen fluid were saved for histamine analysis. In addition, O'Sullivan (1968) demonstrated that the histamine content of several forages varies markedly with mosisture and temperature conditions. Histamine concentrations of the ruminal fluid samples (four samples per week for five weeks) were determined by a

TABLE I

RUMINAL MOTILITY OF WHEAT PASTURE STOCKERS (SURGICALLY IMPLANTED
PRESSURE TRANSDUCERS), 1975-76

	Mean, Pre-Wheat Pasture		Wheat Pasture					
		DATE:	1-6	1-15	1-19	1-22	1-30[a]	2-14[b]
AMPL., mm Hg	6.7		23.2*	24.9*	23.7*	16.7*	21.7	14.3
FREQ., sec.	33.5		26.5	35.4	32.2	31.6	38.2	45.6

[a,b]2 and 1 steer, respectively.

*Significantly different from mean of pre-wheat pasture period (P < .05).

TABLE II

RUMINAL MOTILITY OF WHEAT PASTURE STOCKERS (WATER-FILLED, BALLOON CANNULAE ATTACHED TO P-1000-A
EXTERNAL PRESSURE TRANSDUCER), 1975-76

	Mean, Post-Wheat Pasture	Wheat Pasture					
		DATE:	3-3	3-10	3-17	3-26	4-2
AMPL., mm Hg	12.9		18.6	22.7*	23.5*	25.2*	17.9
FREQ., sec.	32.2		40.0	38.4	41.4*	36.6	28.3

*Significantly different from mean of post-wheat pasture period (P < .05).

fluorometric procedure (Shore et al., 1959; Hakansan et al., 1972).
The samples from December 9, 1975, to January 15, 1976, were analyzed.
Ruminal histamine concentrations ranged from 1.0 to 6.6 µg/100 ml
rumen fluid. Dain et al. (1955) reported signs of acute illness in
overfed sheep when the ruminal histamine concentrations approached
500 µg/100 ml. Based on these preliminary findings, plus the lack of
reduced ruminal motility for that year, it was concluded that ruminal
histamine concentrations were not of sufficient magnitude to alter the
normal ruminal motility behavior of stocker cattle grazed on wheat
pasture. Consequently no further analyses were made.

1976-77 Wheat Pasture Grazing Season

The mean amplitude and frequency of ruminal contractions during
the pre- and post-wheat pasture, and the wheat pasture grazing period
are shown in Tables III and IV, respectively. The mean amplitudes and
frequencies displayed for pre- and post-wheat measurements in Table III
were not different (P > .05) from one another. Large amplitudes in the
range of 40 to 50 mm Hg were also frequently observed during the 1976-
77 grazing season. The amplitude of ruminal contractions was
increased (P < .05) on four dates during the wheat pasture grazing
period, whereas the mean amplitude on March 1, 1977, was decreased
(P < .05). Frequency of ruminal contractions were generally increased,
although significant (P < .05) reductions were observed on March 15,
1977.

The results of the two year study (Table V), expressed as per-
centage changes from the control values infer, that ruminal amplitude
and frequency patterns of stocker calves grazed on winter wheat pasture

TABLE III

RUMINAL MOTILITY OF WHEAT PASTURE STOCKERS (PRE- AND POST-WHEAT), 1976-77

	Pre-Wheat Pasture			Mean, Pre-Wheat Pasture	Post-Wheat Pasture		Mean, Post-Wheat Pasture	Mean, Pre- and Post-Wheat Pasture
DATE:	11-4	11-10	11-11		4-28	5-6		
AMPL., mm Hg	20.0	17.6	17.7	18.4	16.8	13.5	15.2	17.1
FREQ., sec.	27.1	24.3	23.3	24.9	17.3	27.0	22.2	23.8

TABLE IV

RUMINAL MOTILITY OF WHEAT PASTURE STOCKERS, 1976-77

	AMPL., mm Hg	FREQ., SEC.
MEAN, PRE- AND POST-WHEAT PASTURE:	17.1	23.8
WHEAT PASTURE		
11-23	17.3	31.7*
11-29	16.5	29.4
12-7	18.0	32.7*
12-14	15.9	33.8*
12-28	15.9	30.6*
1-4	23.5*	38.0*
1-18	33.5*	33.5*
2-1	25.4*	28.7
2-8	15.2	29.6
2-15	12.4	34.4*
2-22	26.6*	22.0
3-1	11.0*	35.4*
3-8	15.9	21.9
3-15	15.1	15.8*

*Significantly different from mean of pre- and post-wheat pasture periods (P < .05).

TABLE V

MEAN AMPLITUDE AND FREQUENCY OF RUMINAL CONTRACTIONS

| | Amplitude, mm Hg | | Frequency, Sec. | |
	Control	Wheat Pasture	Control	Wheat Pasture
1975-76				
Implanted Pressure Transducers	6.7	22.12 (+230)[a]	33.5	24.8 (-6)[a]
Water-filled Balloon Cannulae	12.9	21.58 (+67)[a]	32.2	36.94 (+15)[a]
AVG	9.8	21.85 (148.5)[a]	32.85	30.87 (+4.5)[a]
1976-77				
	17.1	18.69 (+9)	23.8	29.82 (+25)

[a]Parenthetical numbers represent the percentage increase of wheat pasture from the control.

are increased. The data indicate that wheat pasture bloat of stockers
does not occur secondarily to a reduced ruminal motility.

CHAPTER IV

BLOAT POTENTIAL OF WHEAT PASTURE AND ITS

RELATIONSHIP TO CHEMICAL COMPONENTS OF

WHEAT FORAGE AND RUMEN FLUID

Summary

Ruminal fluid foam stability, expansion and strength, as indices of

the likelihood of bloat, of four steer calves were measured at weekly

intervals during the 1975-76, 1976-77 and 1977-78 wheat pasture grazing

periods. Ruminal fluid viscosity (1976-77) was also measured, as a

possible alternative to the ruminal fluid foam measurements from January

18, 1977, to March 15, 1977. Additional analyses were conducted for

wheat forage chemical components in order to observe changes in concen-

tration during the three separate grazing periods. Soluble nitrogen

fractions (1977-78) of rumen fluid were also examined. Coefficients of

determination (R^2) of the regression of ruminal fluid foam stability,

expansion and strength on single or multiple chemical components of

wheat forage and rumen fluid were determined by using the statistical

analysis system all possible regression models program. The ruminal

fluid foam parameters changed ($P < .05$) during the 1975-76 and 1976-77

wheat pasture grazing seasons, but the high and low yearly values did

not occur at the same times across years. Ruminal fluid viscosity

showed no significant differences ($P > .05$) over time. Chemical com-

ponent concentrations of wheat forage also changed ($P < .05$) during

all three wheat grazing years. The soluble nitrogen fractions of rumen fluid also changed ($P < .05$) during 1977-78. Maximum variation in foam stability, expansion and strength measurements accounted for by the forage chemical components were, respectively: 1975-76; 9.2% (dry matter, soluble carbohydrates, crude protein, foam-stabilizing protein), 7.8% (dry matter, soluble carbohydrates crude protein, foam-stabilizing protein) and 33.4% (dry matter, soluble carbohydrates, crude protein, foam-stabilizing protein); 1976-77; 50.1% (dry matter, neutral-detergent fiber, crude protein, total soluble nitrogen, soluble protein nitrogen), 39.3% (dry matter, neutral-detergent fiber, crude protein, total soluble nitrogen, soluble protein nitrogen), 49.3% (dry matter, neutral-detergent fiber, crude protein, total soluble nitrogen, soluble non-protein nitrogen); and 1977-78; 34.7% (dry matter, neutral-detergent fiber, crude protein, total soluble nitrogen, soluble non-protein nitrogen), 22.0% (neutral-detergent fiber, crude protein, total soluble nitrogen, soluble protein nitrogen, soluble non-protein nitrogen), 25.2% (dry matter, neutral-detergent fiber, total soluble nitrogen, soluble protein nitrogen, soluble non-protein nitrogen). Maximum variation in ruminal fluid foam stability, expansion and strength accounted for by the total soluble nitrogen, soluble protein nitrogen and soluble non-protein nitrogen fractions of rumen fluid were: 23.0%, 26.8% and 4.7%, respectively.

Introduction

Death losses among stocker cattle grazed on winter wheat pasture in Oklahoma have been reported to be as high as 25,000 head annually

(Johnson, 1973), due to the stocker syndrome. A rapid death seems apparent since very few animals are observed in the process of dying. Due to the nature of rumen contents, necropsy lessions indicative of antemortem bloat, and the therapeautic effects of poloxalene, frothy bloat is thought to be a contributing factor to the stocker syndrome (Clay, 1973).

A number of forage chemical components have been postulated as important in the production of stable ruminal foams. Bartley and Bassette (1961) concluded that the foaming constituent of cattle grazed on bloat provocative alfalfa pastures was proteinaceous in nature. Howarth et al. (1975) correlated nitrogenous fractions of alfalfa with the incidence of bloat. Correlation coefficients between the incidence of bloat and the nitrogenous fractions were: total nitrogen, $r = .25$ ($P < .05$); insoluble nitrogen, $r = .18$ ($P < .1$); soluble non-protein nitrogen, $r = .10$ ($P < .1$); and soluble protein nitrogen, $r = .34$ ($P < .005$).

Rumbaugh (1969), used the apical 10 cm of a sufficient number of alfalfa stems with attached leaves to conduct a foam test by macerating the selected plant components in a high speed laboratory blender for 4 minutes with 300 mls of pH 5.6 phosphate buffer at room temperature. The contents were then transferred to a 1000 ml graduated cylinder. After two minutes the cylinder was shaken to eliminate any large trapped air pockets and a foam score or stable foam volume of the plant sample was recorded. This researcher reported a correlation coefficient of .18 ($P < .1$) between his foam test with the incidence of bloat. Pressey et al. (1963b) found a correlation coefficient of $r = .56$ ($P < .01$) between bloat incidence in cattle grazing alfalfa and stability of foams generated in vitro.

The objectives of this study were: (1) to measure changes in ruminal fluid foam stability, expansion and strength (as indices of the likelihood of bloat) during the wheat pasture grazing season, (2) to determine the concentrations of specific wheat forage chemical components (believed to be related to the incidence of wheat pasture bloat), and (3) to determine the relationship between concentrations of wheat forage chemical components and the ruminal fluid foam measurements.

Experimental Procedures

Wheat Pasture

The studies were conducted on twenty hectares of wheat pasture at the Oklahoma State University Dairy Cattle Center. Nineteen kg of Triumph 64 seed were sown per hectare on September 8, 1975, and September 9, 1976, and 1977. Prior to drilling, urea (26 kg/ha) was applied, and 9 kg/ha of 18-46-0 fertilizer was included with the seed. There were no additional applications of nitrogen during the grazing periods.

Ruminal Fluid Foam Stability, Expansion
and Strength

Measurements of foam stability, expansion and strength were made on ruminal fluid samples taken from 3 separate sets of 4 fall-weaned rumen cannulated steer calves. Hereford calves were used during the 1975-76 and 1977-78 grazing seasons, while Hereford x Angus calves were used during the 1976-77 grazing period. The length of each

grazing period from which measurements were collected was: (1)
December 23, 1975, to April 1, 1976, (2) December 21, 1976, to March
22, 1977, and (3) November 11, 1977, to April 6, 1978.

During the wheat pasture grazing periods, rumen fluid samples
were taken at weekly intervals. The ruminal fluid samples were
strained through four layers of cheesecloth, and were then centrifuged
at 754 x g for 10 minutes. The supernatant of each sample was then
decanted and used for the measurements of ruminal fluid foam stability,
expansion and strength. The ruminal fluid foam measurements were
made by a modification of the procedure of Mangan (1958). The foam
measurements were obtained by placing a continuous volume of rumen
fluid (40 ml) in a glass column (Figure 4) and passing compressed air
(19-23% oxygen, 77-81% nitrogen) through a fritted glass disc[1] for
10 minutes at a constant pressure (1.95 kg/sq.cm.). The column shown
in Figure 4 (110 cm long, internal diameter of 3.25 cm) was carefully
selected for uniformity of bore. The glass column contains two inlets.
One, through which gas enters, is located 5.5 cm below the fritted
glass disc. The other inlet, through which the rumen fluid enters, is
located 2.5 cm above the fritted glass disc. To avoid parallax errors,
a red line, 4.5 cm above the fritted glass disc, was used to adjust
the amount of rumen fluid to a constant 40 ml volume. The apparatus
was mounted to maintain the column in an exact perpendicular position.
The gas flow was held constant with the aid of an additional pressure
regulator. Foam stabilities were estimated from the slopes (regres-
sion coefficients) of the resulting plots of foam height versus

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[1]Size 30 Course; Kimflow
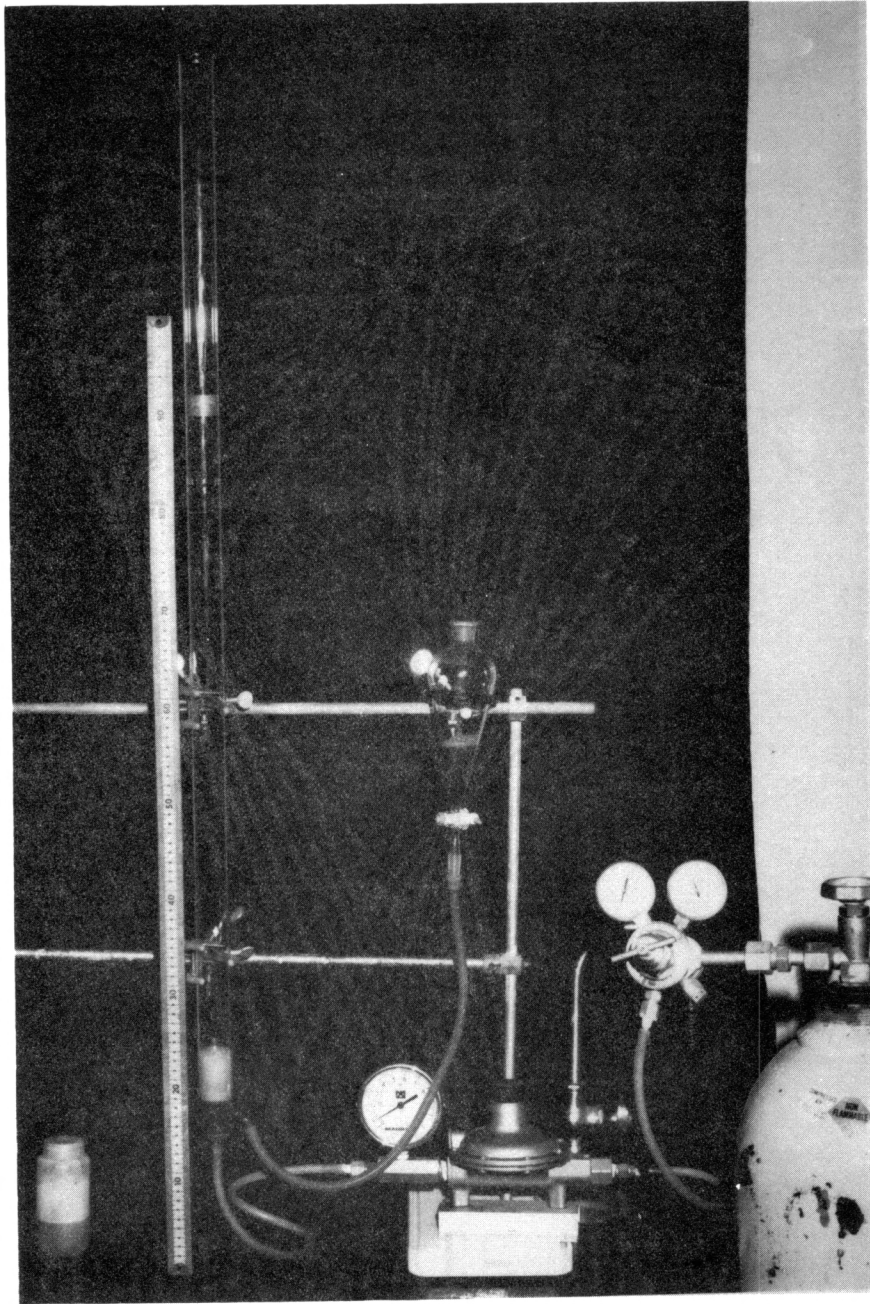
Figure 4. Ruminal Fluid Foam Measuring Apparatus

foaming time. Foam stability was defined as the rate of foam forma-
tion compared to the rate of breakdown, and increased as the magnitude
of the regression coefficients increased. The measurements of foam
expansion and strength were defined as the volume of foam produced from
a given amount of rumen fluid (cm foam/ml fluid) at the end of the 10
minute foaming period, and as the rate of fall (cm/sec) of a perforated
aluminum weight (44 g) through the resultant foams, respectively.

As a possible alternative to the ruminal fluid foam stability,
expansion and strength measurements, ruminal fluid viscosity was
measured from 4 steers at weekly intervals for 8 weeks (January 18,
1977, to March 15, 1977). Studies have shown that ruminal fluid
viscosity is increased in bloated animals, possibly as a result of the
ruptured microbial cell walls and the spillage of cell contents into
the rumen (Clarke, 1965). It has been suggested that lysis of bac-
teria occurs as a result of excess intraruminal pressure and engorge-
ment by certain bacteria of soluble carbohydrates (Clarke, 1965; Jones
and Lyttleton, 1972a). Ruminal fluid viscosity (units of centistrokes)
was determined on strained rumen fluid following analysis with a No.
C-155, size 100 viscometer.

Wheat Forage Chemical Components

From November 23, 1975, to April 1, 1976, 114 samples of wheat
forage for 19 weeks, 6 samples per week, were collected. Each
sample represented clippings of wheat pasture from 1 of 6 randomly
assigned areas in the test plot. The samples were immediately frozen
in a liquid nitrogen tank (-196 C), and then ground with dry ice in a

Wiley Mill, through a 2 mm screen. After grinding, the samples were stored in plastic bags in a freezer (-20 C) until chemical analyses were conducted. The plastic bags were left unsealed overnight to permit the dry ice residue to evaporate prior to being sealed. Table VI shows the specific chemical components examined and the sampling time intervals at which they were analyzed.

During November 4, 1976, to March 22, 1977, samples of wheat forage for 19 weeks were collected in the same manner as the previous year and represented the same random clipping procedure for obtaining 6 individual represenative samples. The forage samples were frozen (-20 C) immediately after collection and ground with dry ice through a 2 mm screen and stored as described for the 1975-76 grazing period. Table VI describes the specific chemical components examined and the time intervals at which they were analyzed. The 1977-78 wheat grazing season began on November 11, 1977, and continued until April 6, 1978. During this length of time, 8 weeks of forage samples were collected from only 4 randomly assigned areas of the test plot, from which 4 representative samples were taken. Samples from this grazing season were analyzed for dry matter content immediately after being collected. These field dry matter evaluations were determined in a Despatch Batch oven at a temperature of 55 C. These samples were then ground in a Wiley Mill through a 2 mm screen. Table VI describes the specific chemical components examined and the time intervals at which they were analyzed.

Neutral-detergent fiber analyses were conducted according to the procedure of Goering and Van Soest (1970). Crude protein content of

TABLE VI

WHEAT FORAGE CHEMICAL COMPONENTS ANALYZED DURING THE
VARIOUS WHEAT PASTURE GRAZING SEASONS

Chemical Component	Year		
	1975-76	1976-77	1977-78
Dry matter	x^a	x^a	x^b
Neutral-detergent fiber		x^b	x^b
Soluble carbohydrates	x^a		
Foam-stabilizing protein	x^a		
Crude protein	x^a	x^a	x^b
Total soluble nitrogen		x^b	x^b
Soluble protein nitrogen		x^b	x^b
Soluble non-protein nitrogen		x^b	x^b

[a] Samples analyzed at one week intervals.

[b] Samples analyzed at two week intervals.

the forage samples was determined.by the macro-kjeldahl method (A.O.A.C., 1975). Analyses of the soluble nitrogen fractions were conducted using a buffer [Na_2HPO_4 (56.5 g/ℓ), NaH_2PO_4 (54.5 g/ℓ), KCl (21.5 g/ℓ), NaCl (21.5 g/ℓ), $MgSO_4 \cdot 7H_2O$ (5.82 g/ℓ), K_2SO_4 (7.5 g/ℓ), from the "Ohio" in vitro fermentation media (Johnson, 1969). Two or 0.5 g of wet or dry forage, respectively, were incubated in 125 ml buffer (pH 6.5) at 39 C in a shaking water bath for one hour. The solution was then filtered through Whatman #4 filter paper. Fifty ml aliquots of the filtrates were then analyzed for total soluble nitrogen by the macro-kjeldahl method (A.O.A.C., 1975); a blank consisted of 50 ml of buffer. Total soluble non-protein nitrogen concentrations were determined by deproteinizing 25 ml of the filtrate with 5 ml 1.07 $\underline{N}$ H_2SO_4 and 5 ml 10% sodium tungstate. This solution was mixed in centrifuge tubes and allowed to settle overnight in a refrigerator (5 C). The following morning the samples were centrifuged at 12,062 x g for 10 minutes, and 25 ml of the supernatant fluid was analyzed for total nitrogen by the macro-kjeldahl method (A.O.A.C., 1975). Twenty-five ml of a solution of 25 ml buffer, 5 ml 1.07 $\underline{N}$ H_2SO_4 and 5 ml of 10% sodium tungstate was used as a blank. Forage soluble protein nitrogen concentrations were calculated by the difference in nitrogen content of total soluble nitrogen and soluble non-protein nitrogen concentrations.

The phenol-sulfuric acid procedure (Johnson et al., 1966) was used to determine the soluble carbohydrate content of wheat forage samples.

Ribulose-1,5-diphosphate carboxylase (RuDP carboxylase or foam-stabilizing protein) was assayed, discontinuously, as the amount of radioactive, acid-stable product (3-phosphoglycerate) produced during a 20-minute reaction period at 25 C in a shaking water bath (Chu and Bassham, 1973). Each reaction mixture of 0.4 ml total volume contained ribulose-1,5-diphosphate, (0.5 mM), $MgCl_2 \cdot 6H_2O$ (10 mM), $NaH^{14}CO_3$[2] (.29 mM), and the enzyme preparation (0.1 ml) in 0.1 M tris-HCl buffer, pH 7.8. Enzyme preparations were prepared by homogenizing[3] 5 g of wheat forage in 9 volumes of cold water. Homogenization in tris-HCl buffer, pH 7.8, versus water did not affect enzyme activity. The homogenates were then centrifuged for 10 minutes at 1086 x g, and the supernatant fluid was used as the enzyme preparations. At the end of the reaction period, 0.1 ml of concentrated glacial acetic acid was added to stop the reaction. The reaction vials were then flushed with nitrogen at room temperature to dryness. Water (0.5 ml) was added to each reaction vial to dissolve the residue. Ten ml of scintillation solution was added to each vial, and radioactivity was determined by counting in a liquid scintillation counter. Blanks to correct for any 3-phosphoglycerate produced non-enzymatically were prepared by substitution of buffer for enzyme preparation. One unit of RuDP carboxylase activity was defined as that amount of enzyme which catalyzed the cleavage of 1 μmole of ribulose diphosphate to 2 μmoles of 3-phosphoglycerate per minute at 25 C.

Plots of radioactivity (counts/minute) versus (1) .05 to .25 ml of enzyme preparation (.05 ml increments) at a fixed reaction time of

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[2] Specific activity: 43.3 mCi/mmole, New England Nuclear; 549 Albany Street; Boston, Mass. 02118

[3] Sorvall Omni-mixer.

20 minutes, and (2) reaction times of 5 to 30 minutes (5 minute incre-
ments) at a fixed amount of enzyme preparation (0.1 ml) were linear.

Standard vials containing 1 to 20 units x 10^{-4} of standard
RuDP carboxylase[4] in place of the wheat forage enzyme preparation were
included in each set of assays. The regression of radioactivity versus
units of standard enzyme was used to convert radioactivity resulting
from wheat forage enzyme preparation to units of RuDP carboxylase
activity.

Ruminal Fluid Analyses

Ruminal fluid pH was of interest since the acidity of the rumen
affects the formation of stable foams (Mangan, 1959; Wright, 1959).
Ruminal fluid pHs were measured during each of the three grazing sea-
sons with a Corning Model 12 Research pH meter.

Ruminal ammonia concentrations of the steers grazed on wheat
pasture were also analyzed for each of the three grazing periods.
Fifty ml of the strained ruminal fluid were acidified with 1 ml of 20%
H_2SO_4, sealed in a plastic container and immediately placed on ice.
The samples were then transported to the laboratory and analyzed for
ammonia by the magnesium oxide distillation step of the macro-kjeldahl
procedure (A.O.A.C., 1975).

During the 1977-78 grazing period, additional aliquots of strained
ruminal fluid were collected and analyzed for total soluble nitrogen,
soluble protein nitrogen and soluble non-protein nitrogen. The samples
were centrifuged at 27,138 x g for 20 minutes, and 25 ml aliquots of

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[4]Type I; Partially purified powder from spinach; Sigma Chemical
Company; 3500 DeKalb Street; St. Louis, Missouri 63118.

the supernatant fluids were analyzed for total soluble nitrogen by the macro-kjeldahl procedure (A.O.A.C., 1975). The supernatant fluids (12.5 ml aliquots) were then deproteinized with 2.5 ml of 1.07 $\underline{N}$ H_2SO_4 and 2.5 ml of 10% sodium tungstate. These solutions were then placed in the refrigerator (5 C) overnight. The samples were centrifuged at 12,062 x g the following morning, and 12.5 ml aliquots of the supernatant fluids were analyzed for total nitrogen. Soluble protein nitrogen was calculated by the difference between the total soluble nitrogen and soluble non-protein nitrogen concentrations.

Statistical Analyses

All ruminal fluid measurements were analyzed by analysis of variance procedures for a randomized complete-block design with steers as blocks and time as treatment. Forage data were analyzed as a completely randomized design with time as treatment. Differences among treatment means for both the ruminal fluid and forage data were tested for sifnificance by an LSD protected by a preliminary F test (Steel and Torrie, 1960). The error mean squares for the ruminal fluid and forage data were date x steer and date x forage, respectively, and were used for calculating the F values.

The statistical analysis system all possible regression models program was used to obtain coefficients of determination from the regression of ruminal fluid foam measurements on wheat forage chemical chemical components. The following model was used:

$$Y = \hat{\alpha} + \hat{\beta}_1 X_1 + \hat{\beta}_2 X_2 + \hat{\beta}_3 X_3 + \hat{\beta}_4 X_4 + \hat{\beta}_5 X_5 + \hat{\beta}_6 X_6$$

where X_1, X_2, X_3, X_4, X_5 and X_6 represent the independent forage variables that were included in the regression model. The dependent variables for each week were considered to be individual steer observations and were regressed on the means of each of the independent variables for the respective week. Separate equations were determined for each of the three dependent variables:

Y_1 = Foam stability

Y_2 = Foam expansion

Y_3 = Foam strength

Results and Discussion

Ruminal Fluid Foam Stability, Expansion and Strength

Ruminal fluid foam stability, expansion and strength measurements of samples collected during the 1975-76, 1976-77 and 1977-78 wheat pasture grazing seasons are shown in Tables VII, VIII and IX, respectively. The initial foam stability values (12-23-75, 12-21-76, 11-11-77) for each year were assigned values of 100%, and the remaining measures of foam stability were expressed as a percentage of these initial values. Significant differences ($P < .05$) were observed in the measurements of ruminal fluid foam stability, expansion and strength at the different sampling times during 1975-76 and 1976-77 grazing seasons.

During the 1975-76 wheat pasture period, the largest foam stability value (420% increase in the initial value) was observed on March 11, 1976. This increase coincided with a weekly minimum-maximum average temperature of 0-13.5 C and 2.6 cm of precipitation during the previous

TABLE VII

RUMINAL FLUID FOAM STABILITY, EXPANSION AND STRENGTH MEASUREMENTS (1975-76)

Date	n	Stability		Expansion (cm. Foam/ml Fluid)	Strength (cm./sec.)
		Linear Regression Coefficients	Percent of Initial Value		
12-23-75	4	$.396^{bcd}$	100	7.48^{abcd}	----
12-30-75	4	$.268^{bcd}$	68	4.26^{abcd}	----
1-15-76	4	$.303^{bcd}$	77	6.25^{abcd}	1.92^a
1-22-76	4	$.564^{cd}$	142	9.20^{cd}	2.18^a
1-29-76	4	$.193^{bc}$	49	3.88^{abc}	2.45^a
2-12-76	4	$.061^{b}$	15	2.49^a	2.58^a
2-19-76	4	$.419^{bcd}$	106	7.68^{abcd}	1.92^a
2-26-76	4	$.618^d$	156	9.79^d	3.80^a
3-5-76	4	$.010^a$	3	2.58^{ab}	2.95^a
3-11-76	4	1.665^a	420	26.16^e	10.78^b
3-17-76	4	$.366^{bcd}$	92	7.27^{abcd}	2.65^a
3-25-76	4	$.387^{bcd}$	98	8.28^{bcd}	4.50^a
4-01-76	4	$.393^{bcd}$	99	8.57^{cd}	2.35^a
		S.E. .22		S.E. 3.43	S.E. 2.00

[abc] Means in the same column with common-lettered superscripts are not statistically different (P>.05).

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TABLE VIII

RUMINAL FLUID FOAM STABILITY, EXPANSION, STRENGTH AND VISCOSITY MEASUREMENTS (1976-77)

Date	n	Stability		Expansion (cm Foam/ml Fluid)	Strength (cm/sec)	Viscosity (Centistrokes)
		Linear Regression Coefficients	Percent of Initial Value			
12-21-76	4	$.71^{bc}$	100	1.22^{c}	1.27^{ab}	
12-28-76	4	$.68^{bc}$	95	1.155^{bc}	$.55^{a}$	
1-04-77	4	$.63^{bc}$	88	1.18^{bc}	2.96^{c}	
1-18-77	4	$.60^{abc}$	84	1.14^{bc}	$.60^{ab}$	2.00^{a}
2-01-77	4	$.58^{abc}$	81	1.19^{bc}	1.94^{abc}	2.01^{a}
2-08-77	4	$.60^{abc}$	83	1.22^{c}	1.74^{abc}	1.68^{a}
2-15-77	4	$.89^{c}$	125	1.31^{c}	$.91^{ab}$	1.57^{a}
2-22-77	4	$.57^{abc}$	80	$.75^{abc}$	1.78^{abc}	1.44^{a}
3-01-77	4	$.21^{ab}$	29	$.42^{ab}$	1.08^{ab}	1.63^{a}
3-08-77	4	$.19^{ab}$	27	$.42^{ab}$	1.30^{ab}	2.19^{a}
3-15-77	4	$.06^{a}$	8	$.32^{a}$	2.04^{bc}	1.66^{a}
3-22-77	4	$.28^{ab}$	39	$.57^{abc}$	2.87^{c}	
		S.E. .26		S.E. .38	S.E. .72	S.E. .29

[abc] Means in the same column with common-lettered superscripts are not statistically different (P>.05).

TABLE IX

RUMINAL FLUID FOAM STABILITY, EXPANSION AND STRENGTH MEASUREMENTS (1977-78)

| Date | n | Stability | | Expansion (cm. Foam/ml. Fluid) | Strength (cm./sec.) |
		Linear Regression Coefficients	Percent of Initial Value		
11-11-77	3	.54[a]	100	1.55[a]	.33[a]
11-22-77	4	.81[a]	150	.93[a]	2.45[a]
12-09-77	4	.44[a]	81	.80[a]	1.16[a]
12-21-77	4	.74[a]	137	1.18[a]	.90[a]
1-06-78	4	1.33	246	.92[a]	1.67[a]
3-09-78	3	.17[a]	31	.52[a]	7.32[a]
3-21-78	4	.00[a]	0	.20[a]	2.31[a]
4-06-78	3	.14[a]	26	.54[a]	2.65[a]
S.E. (4.4) .50				.49	1.95
S.E. (4.3) .54				.53	2.10
S.E. (3.3) .56				.57	2.25

[a]Means in the same column with common-lettered superscripts are not statistically different (P>.05).

week (Appendix Figure 12). Foam stabilities of 142 and 156% of the
initial value were observed on January 22 and February 26, 1976,
respectively. The lowest foam stability was observed on March 5, 1976.
An explanation for the marked reduction in foam stability from February
26 to March 5, 1976, is not apparent. However, the crude protein of the
wheat forage, on the dates where ($P < .05$) reductions in foam stability
occurred, was lower ($P < .05$) than that on March 11, 1976, (Appendix
Table XVIII). Although the foam-stabilizing protein concentrations of
wheat forage for March 11, 1976, were significantly lower than the con-
centrations on February 12, 1976, and March 5, 1976. Ruminal fluid
foam expansion and strength measurements also appeared the greatest
on March 11, 1976, while the lowest values coincided with the low foam
stabilities.

The most stable foam observed during the 1976-77 wheat pasture
grazing period occurred on February 15, 1977. This event also coincided
with a weekly minimum-maximum temperature of 0-10 C and 3.2 cm of pre-
cipitation three days prior to sampling (Appendix Figure 12). Both
foam stability and expansion measurements showed similar patterns of
remaining fairly constant until February 15, 1977. The following week,
foam stability decreased to a level similar to that observed on
February 8, 1977, and then decreased even lower for the remainder of
the grazing period (Table VIII). Foam expansion decreased in a similar
manner until March 22, 1977. A similar decrease in crude protein
content of the wheat pasture was also observed (Appendix Table XIX).

No change over time ($P > .05$) was observed in the ruminal fluid
viscosity measurements (Table VIII). The regression of ruminal fluid

viscosity measurements resulted in correlation coefficients of $r = -.14$, $r = .22$ and $r = - .21$, respectively.

Ruminal fluid foam measurements during 1977-78 were limited in number due to an unusually severe winter. As a result of snow and/or ice cover on the wheat pasture from the beginning of January to the end of February, no measurements were made. The 1977-78 ruminal fluid foam data represented approximately two-thirds the observations taken during the two previous years. Differences among the ruminal fluid foam measurements were not different $(P > .05)$, although the wheat forage chemical components did show $(P < .05)$ difference throughout the grazing period (Appendix Table XX). Appendix Table XXI depicts the analysis of variance for each of the ruminal fluid foam parameters for the 1977-78 grazing period.

In general , as foam stability increased or decreased, concomitant increases or decreases occurred in foam expansion. Foam strength appeared to be more variable than either of the other two foam measurements. The effect of yearly environmental variations in the pattern of change in ruminal fluid foam stability showed marked changes across years (Figure 5). These data further suggest that the abrupt changes in foam stability, expansion and strength are consistent with the sporadic incidence of frothy bloat.

Wheat Forage Chemical Components

The concentration of all wheat forage chemical components measured during the three year study changed $(P < .05)$ with time during each of the three years (Appendix Tables XVII, XIX, XX). The changes in wheat forage dry matter, neutral-detergent fiber (1976-77 and
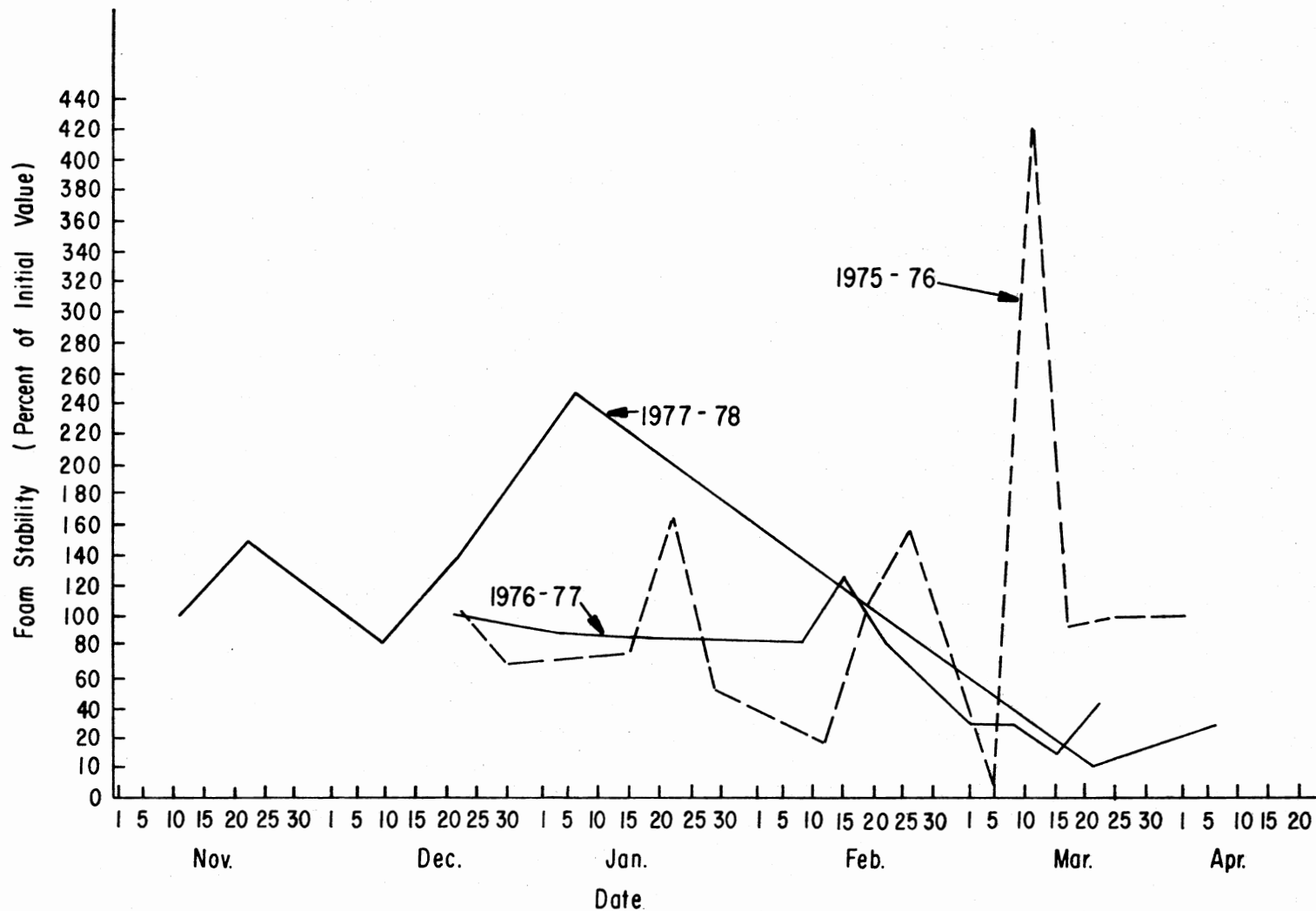
Figure 5. Year to Year Variation in Ruminal Fluid Foam Stability

1977-78 grazing periods only) and crude protein concentrations (which may reflect forage maturity and the incidence of bloat) are illustrated for each of the three years in Figures 6, 7, and 8 respectively. In general, an increase in the crude protein content coincided with a decrease in dry matter and neutral-detergent fiber content, during periods of rapid forage growth (fall and spring), when moisture was more plentiful and the days were warmer (Appendix Figure 12). The large early increases in dry matter and neutral-detergent fiber concentrations in wheat forage and the concomitant decrease in the forage crude protein content during the Fall of 1976 are probably atypical and reflect the effect of the extremely dry and cold growing conditions for wheat pasture. Figures 9, 10 and 11 depict the year to year variation in wheat forage dry matter, neutral-detergent fiber and crude protein content, and demonstrate the difficulty and comparing forage growth patterns across years.

Coefficients of determination (R^2) for the regression of ruminal fluid foam stability, expansion, or strength on chemical components of wheat forage during the 1975-76, 1976-77 and 1977-78 wheat pasture grazing periods are shown in Tables X, XI and XII, respectively. Although the R^2 values are quite low, it is evident that two to three of the forage components accounted for the majority of the variability in each of the foam measurements for each year. For example, forage dry matter and crude protein (R^2 = .485) content accounted for 98% of the maximum variation (R^2 = .493) which was accounted for in foam strength during the 1976-77 wheat pasture period. The independent variables listed in Tables X, XI and XII are believed to be related to the incidence of bloat. Howarth et al. (1975) reported correlation
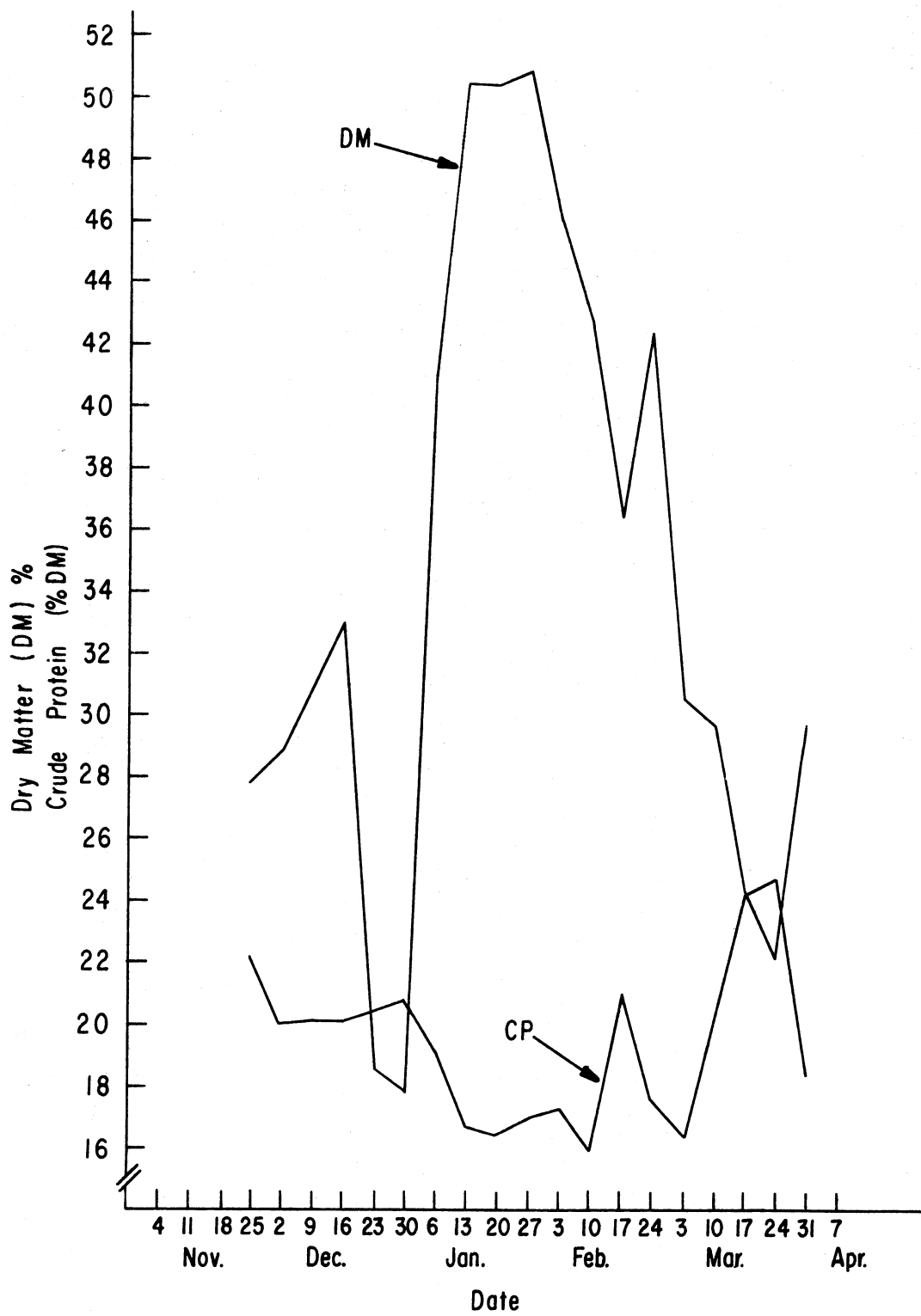
Figure 6. Dry Matter and Crude Protein Content of Wheat
Forage 1975-76
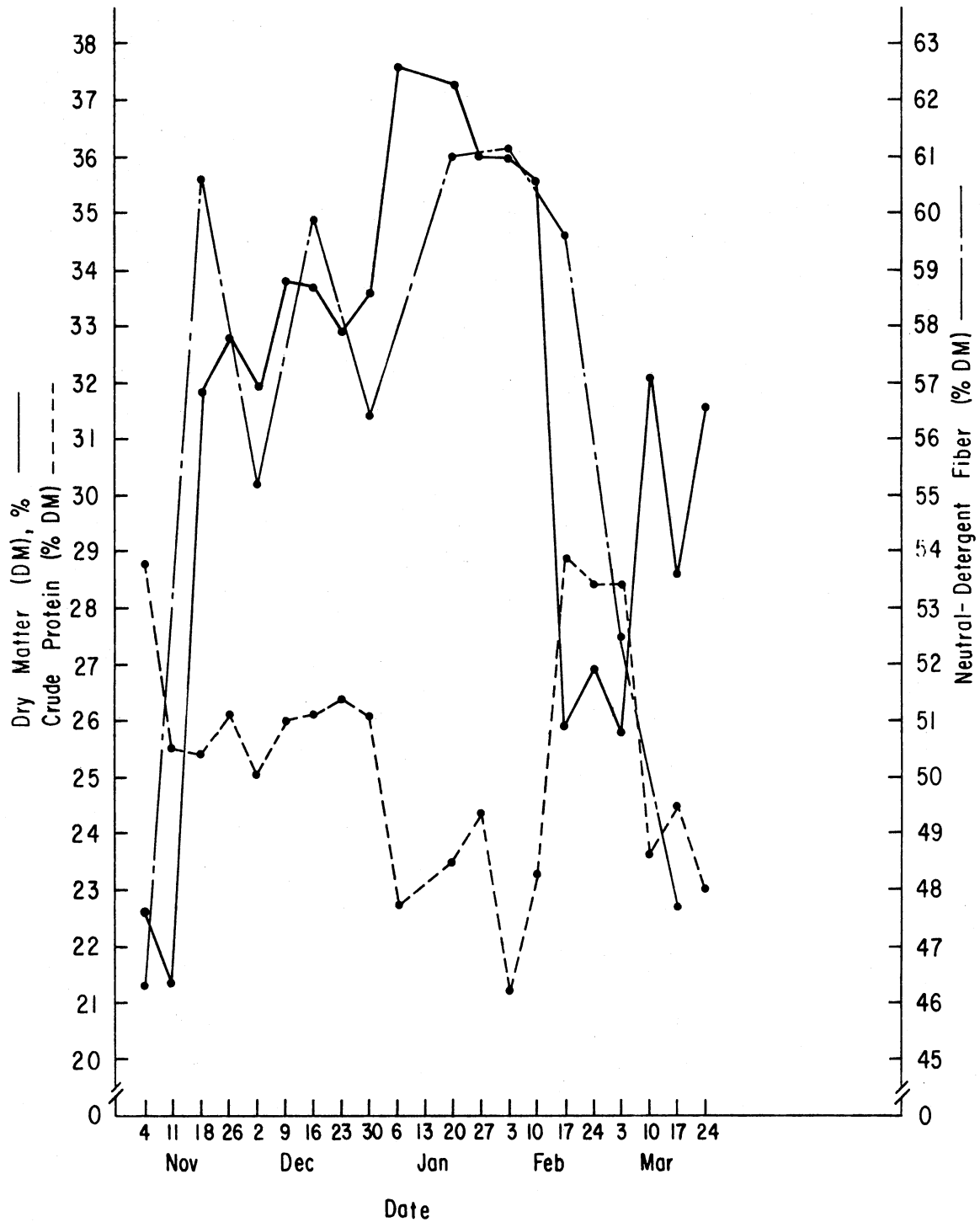
Figure 7. Dry Matter, Neutral-detergent Fiber and Crude
Protein Content of Wheat Forage, 1976-77
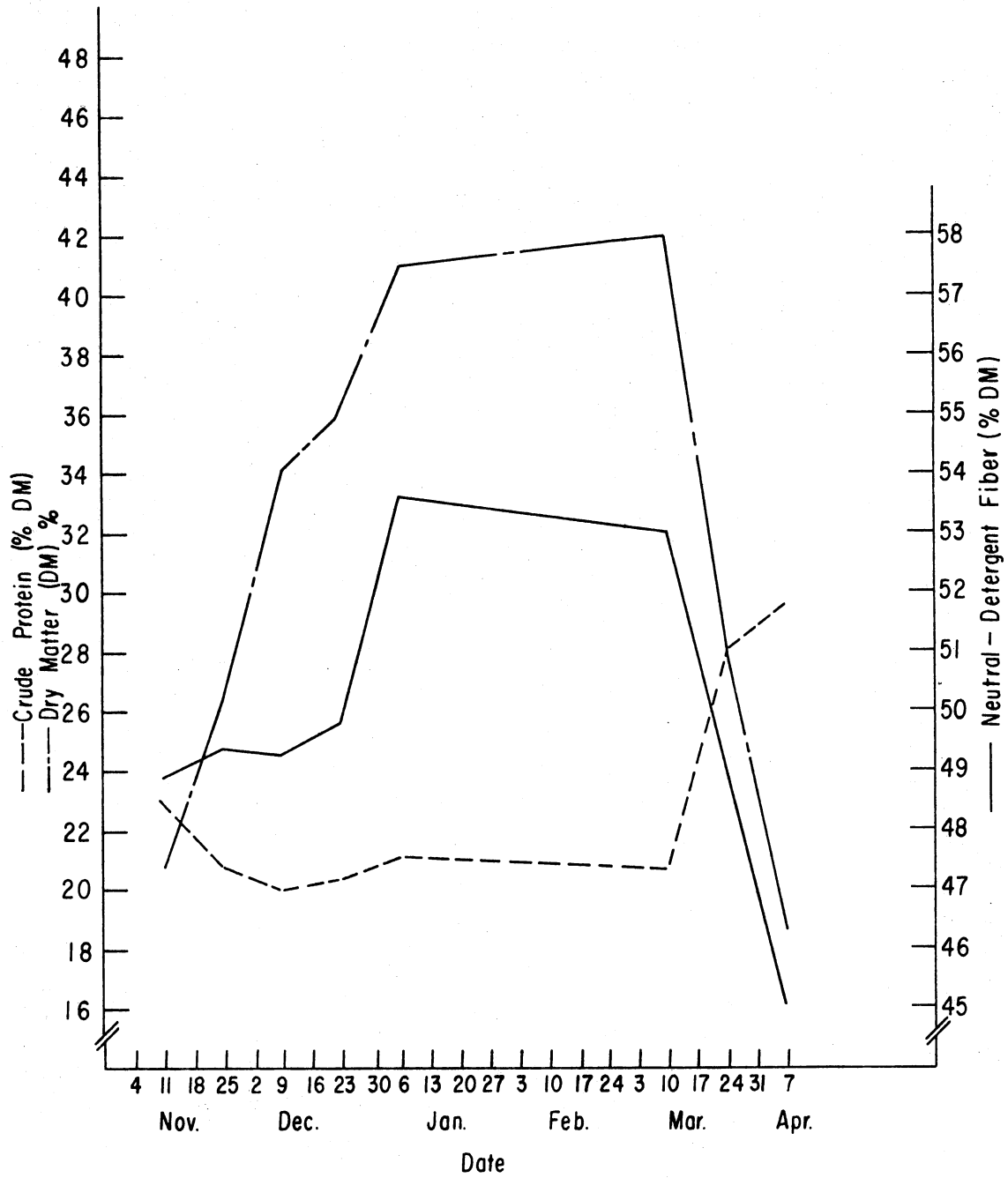
Figure 8. Dry Matter, Neutral-detergent Fiber and Crude
Protein Content of Wheat Forage, 1977-78
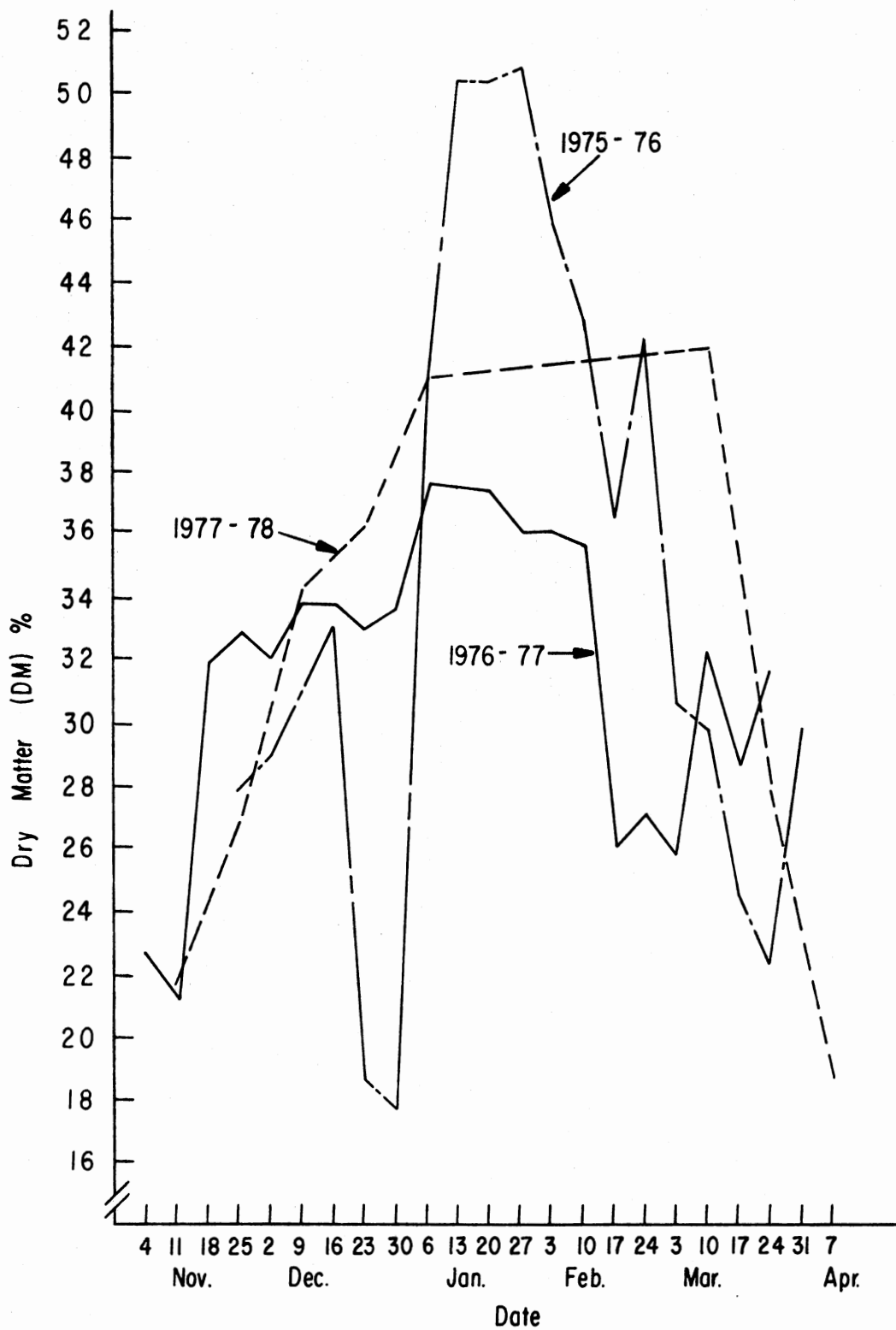
Figure 9.   Year to Year Variation in Dry Matter Content of
Wheat Forage
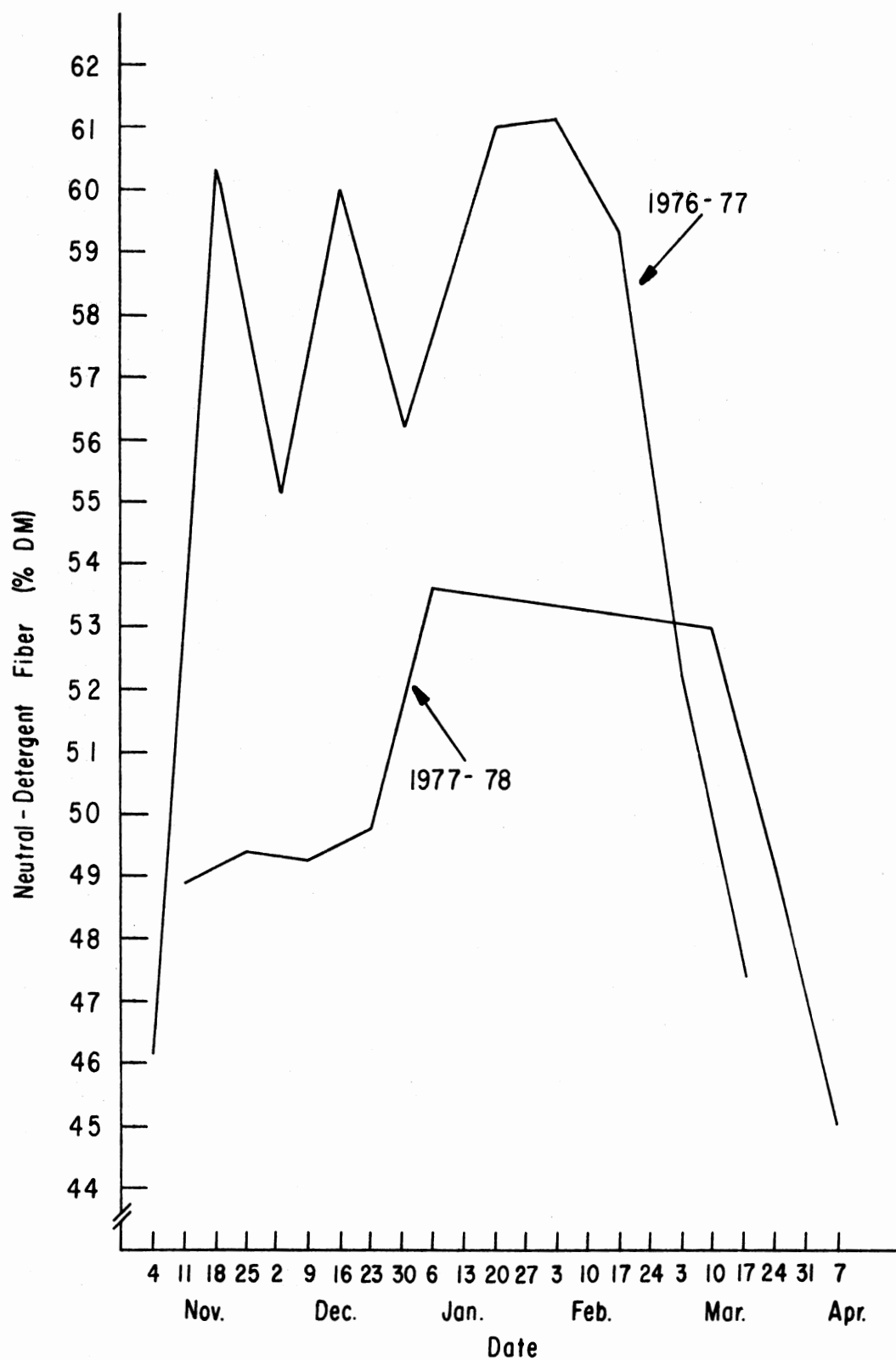
Figure 10.   Year to Year Variation in Neutral-detergent
Fiber Content of Wheat Forage
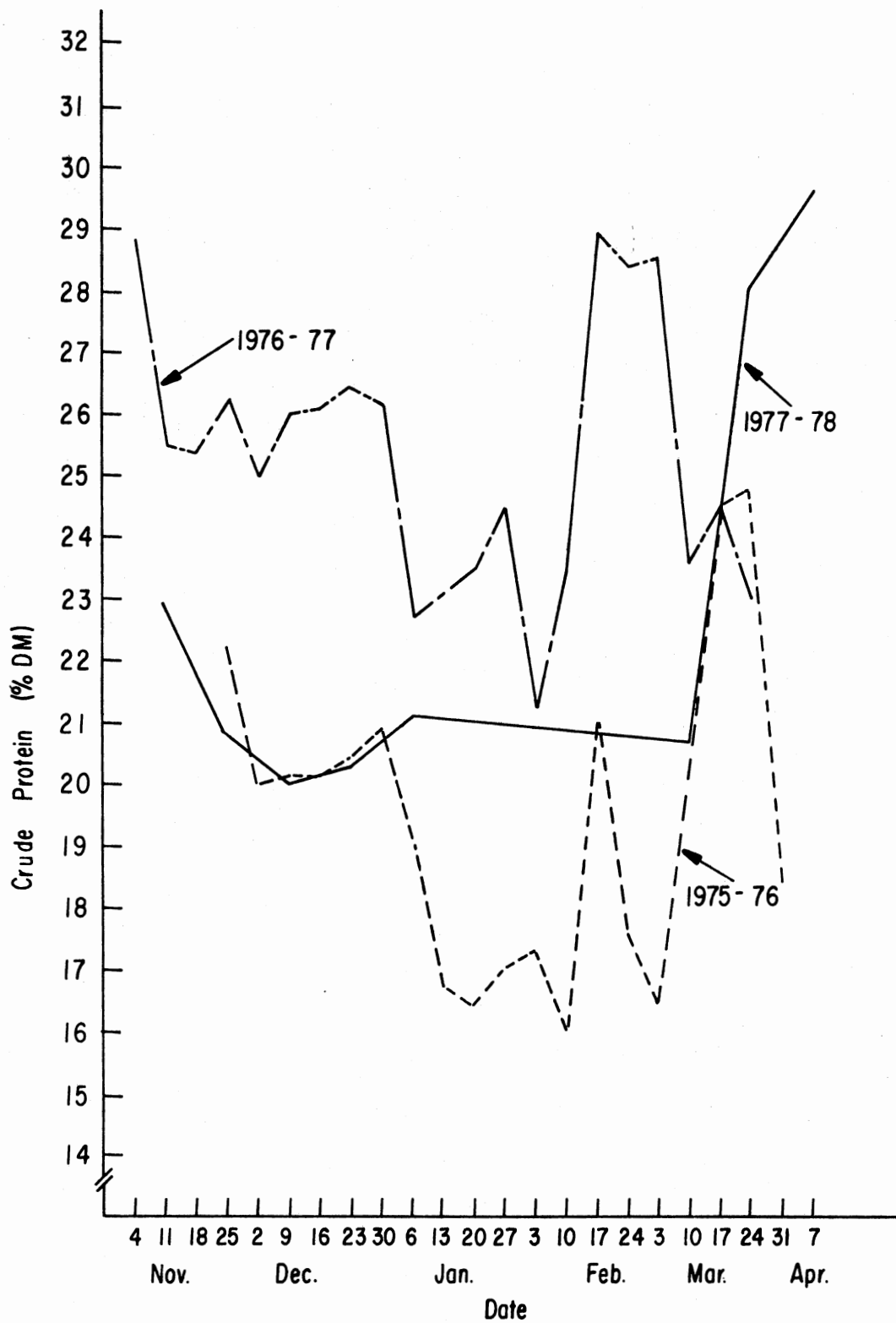
Figure 11. Year to Year Variation in Crude Protent Content of Wheat Forage

TABLE X

COEFFICIENTS OF DETERMINATION (R^2) FOR REGRESSION OF RUMINAL FLUID FOAM STABILITY, EXPANSION OR STRENGTH ON CHEMICAL COMPONENTS OF WHEAT FORAGE (1975-76)

Dependent Variable	Number of Independent Variables	Dry Matter	Soluble Carbohydrate	Crude Protein	FSP[a]	FSP[b]	R^2 Value
Foam Stability	1					x	.025
	2			x		x	.062
	3	x		x		x	.079
	4	x		x	x	x	.085
	5	x	x	x	x	x	.092
Foam Expansion	1			x			.031
	2			x		x	.056
	3	x		x		x	.071
	4	x		x	x	x	.076
	5	x	x	x	x	x	.078
Foam Strength	1		x				.083
	2	x				x	.318*
	3	x		x		x	.326*
	4	x	x	x		x	.328*
	5	x	x	x	x	x	.334*

[a] Foam-stabilizing protein (units x 10^{-4}/gm forage dry matter).

[b] Foam-stabilizing protein (units x 10^{-4}/mg protein).

*($P < .05$).

TABLE XI

COEFFICIENTS OF DETERMINATION (R^2) FOR REGRESSION OF RUMINAL
FLUID FOAM STABILITY, EXPANSION OR STRENGTH ON CHEMICAL
COMPONENTS OF WHEAT FORAGE (1976-77)

Dependent Variable	Number of Independent Variables	Dry Matter	NDF[a]	Crude Protein	Total Soluble Nitrogen	Soluble Protein Nitrogen	Soluble NPN[b]	R^2 Value
Foam Stability	1		x					.360*
	2		x	x				.418*
	3		x	x		x		.459*
	4		x	x	x		x	.487*
	5	x	x	x	x	x		.501*
Foam Expansion	1		x					.332*
	2		x				x	.343*
	3		x	x		x		.367*
	4		x	x	x		x	.382*
	5	x	x	x	x	x		.393
Foam Strength	1					x		.232*
	2	x		x				.485*
	3				x	x	x	.488*
	4	x	x	x		x		.492*
	5	x	x	x	x		x	.493*

[a]Neutral-detergent fiber.

[b]Non-protein nitrogen.

*($P < .05$).

TABLE XII

COEFFICIENTS OF DETERMINATION (R^2) FOR REGRESSION OF RUMINAL
FLUID FOAM STABILITY, EXPANSION AND STRENGTH ON CHEMICAL
COMPONENTS OF WHEAT FORAGE (1977-78)

Dependent Variable	Number of Independent Variables	Dry Matter	NDF[a]	Crude Protein	Total Soluble Nitrogen	Soluble Protein Nitrogen	Soluble NPN[b]	R^2 Value
Foam Stability	1			x				.132
	2				x		x	.213*
	3				x	x	x	.300*
	4	x	x	x			x	.336*
	5	x	x	x	x		x	.347
Foam Expansion	1					x		.114
	2	x		x				.184
	3	x	x	x				.191
	4			x	x	x	x	.210
	5		x	x	x	x	x	.220
Foam Strength	1					x		.150*
	2	x			x			.178
	3	x				x	x	.246
	4		x	x		x	x	.251
	5	x	x		x	x	x	.252

[a]Neutral-detergent fiber.

[b]Non-protein nitrogen.

*($P < .05$).

coefficients between the incidence of bloat and total nitrogen, soluble

protein nitrogen and soluble non-protein nitrogen of fresh alfalfa to be

$r = .25$ (P < .05), $r = .34$ (P < .005) and $r = .1$ (P > .1), respectively.

Horn et al. (1976) reported less (P < .05) dry matter and neutral-

detergent fiber content of forage samples from bloat provocative wheat

pastures. They further indicated that the concentrations of crude

protein, total soluble nitrogen, soluble protein nitrogen and soluble

non-protein nitrogen fractions of wheat forage samples from bloat

provocative pastures were all increased (P < .05).

Ruminal Fluid Analyses

Ruminal fluid pH changed significantly during the grazing period

of each year (P < .05, 1975-76 and 1976-77; P < .08, 1977-78). Mean

ruminal pHs for the various sampling dates are shown in Table XIII.

The pH ranges for the 1975-76, 1976-77 and 1977-78 wheat pasture graz-

ing seasons were 5.9 to 6.4, 6.0 to 6.8, and 6.3 to 6.7, respectively.

In general, pH values remained well above 6.0, and consequently out of

the pH range of 5.4 to 5.7 for maximum foam strength of leaf cyto-

plasmic isolates of red clover (Laby, 1969; Mangan, 1959). Jones

and Lyttleton (1969) found maximum foam strength for white clover

protein to be near pH 5.8. Although Mangan (1959) did show maximum

strengths at pH values ranging from 5.4 to 6.0 for foams from red-clover

cytoplasmic protein. Buckingham (1970) reported marked decreases in

foam strength of red-clover cytoplasmic proteins above pH 6.0 and

below pH 5.0.

TABLE XIII

RUMEN pH OF STOCKER CATTLE GRAZING WHEAT FORAGE

				Year				
1976-76			1976-77			1977-78		
Date	n		Date	n		Date	n	
12-9	4	6.34^{cd}	12-21	4	6.15^{ab}	11-11	3	6.37^{a}
12-16	4	6.19^{bcd}	12-28	4	6.07^{ab}	11-22	4	6.36^{a}
12-23	4	6.10^{abc}	1-4	4	6.44^{c}	12-9	4	6.62^{b}
12-30	4	6.42^{d}	1-18	4	6.77^{d}	12-21	4	6.62^{b}
1-15	4	6.00^{ab}	2-1	4	6.29^{bc}	1-6	4	6.52^{ab}
1-22	4	6.25^{bcd}	2-8	4	6.00^{a}	3-9	3	6.72^{b}
1-29	4	6.34^{cd}	2-15	4	6.21^{abc}	3-21	4	6.34^{a}
2-12	4	6.40^{d}	2-22	4	6.16^{ab}	4-6	3	6.29^{a}
2-19	4	6.21^{bcd}	3-1	4	6.04^{ab}			
2-26	4	6.08^{abc}	3-8	4	6.22^{abc}			
3-5	4	6.30^{cd}	3-15	4	6.16^{ab}			
3-11	4	6.08^{abc}	3-22	4	6.01^{a}			
3-17	4	5.90^{a}						
3-25	4	5.88^{a}						
4-1	4	5.98^{ab}						
	S.E.	.17		S.E.	.12		S.E.(4,4)	.13
							S.E.(4,3)	.14
							S.E.(3,3)	.14

[abcd] Means in the same column with common-lettered superscripts are not statistically different (P > .05).

Due to reports of high protein content of wheat forage (Horn et al., 1976), and that 17 to 33% of the nitrogen is in the form of non-protein nitrogen (Johnson et al., 1974), it has been suggested that ammonia toxicity may be a etiological factor in the stocker syndrome. Ruminal fluid ammonia concentrations changed significantly (P < .05) during the grazing period of each grazing year (Table XIV). The ammonia concentration ranges for the 1975-76, 1976-77 and 1977-78 grazing seasons were 24.7 to 53, 26.2 to 76.6, and 28.2 to 73.9 respectively. Therefore, it was concluded that the postulated association between ammonia toxicity and the stocker syndrome did not exist in the trials conducted.

The soluble nitrogen components of rumen fluid are listed in Table XV. All of the components showed significant differences (P < .05) over time. The soluble nitrogen components of rumen fluid were studied in an attempt to account for more of the variability observed in the ruminal fluid foam measurements (Table XVI). However, ruminal fluid soluble nitrogen components, in general, accounted for smaller proportions of the total variability of the foam measurements than did the soluble nitrogen components of the wheat forage (Table XII). Although considerably larger values were obtained in attempting to regress each of the rumen fluid soluble nitrogen components on multiple wheat forage chemical components (Table XVII).

TABLE XIV

RUMEN AMMONIA NITROGEN CONCENTRATIONS (mg/100 ml)
OF STOCKER CATTLE GRAZING WHEAT FORAGE

		Year						
1975-76			1976-77			1977-78		
Date	n		Date	n		Date	n	
12-9	4	29.75^{abcd}	11-29	4	45.50^{cde}	11-11	3	40.79^{bc}
12-16	4	28.81^{abcd}	12-14	4	41.84^{bcd}	11-22	4	44.35^{c}
12-23	4	38.13^{ef}	12-21	4	46.12^{de}	12-9	4	33.57^{ab}
12-30	4	25.38^{ab}	12-28	4	37.18^{bcd}	12-21	4	37.44^{bc}
1-15	4	28.69^{abcd}	1-4	4	39.31^{bcd}	1-6	4	33.66^{ab}
1-22	4	24.69^{a}	1-18	4	35.43^{b}	3-9	3	28.16^{a}
1-29	4	26.44^{abc}	2-1	4	26.15^{a}	3-21	4	73.90^{e}
2-12	4	33.00^{cde}	2-8	4	36.62^{bc}	4-6	3	54.92^{d}
2-19	4	42.38^{fg}	2-15	4	58.25^{fg}			
2-26	4	35.25^{def}	2-22	4	76.65^{h}			
3-5	4	53.00^{h}	3-1	4	66.40^{g}			
3-4	4	50.19^{h}	3-8	4	59.93^{fg}			
3-17	4	48.06^{gh}	3-15	4	52.75^{ef}			
3-25	4	31.31^{abcd}	3-22	4	43.93^{bcde}			
4-1	4	32.19^{bcde}						
	S.E.	4.42		S.E.	4.51		S.E.(4,4)4.14	
							S.E.(4,3)4.47	
							S.E.(3,3)4.78	

^{abcdefgh}Means in the same column with common-lettered superscripts
are not statistically different (P > .05).

TABLE XV

RUMINAL FLUID SOLUBLE NITROGEN COMPONENTS[1]
(1977-78)

Date	n	Total Soluble Nitrogen	Soluble Protein Nitrogen	Soluble Non-protein Nitrogen
11-11-77	3	52.7^{bc}	17.4^{bc}	35.3^{b}
11-22-77	4	39.7^{abc}	5.3^{a}	34.4^{b}
12-09-77	4	34.3^{a}	7.4^{a}	26.9^{ab}
12-21-77	4	43.9^{abc}	10.9^{ab}	32.5^{ab}
1-06-78	4	37.5^{ab}	8.3^{a}	29.1^{ab}
3-09-78	3	30.9^{a}	8.8^{a}	22.1^{a}
3-21-78	4	87.1^{d}	23.9^{c}	63.2^{c}
4-06-78	3	53.9^{c}	17.0^{b}	36.9^{b}
S.E. (4,4)		6.81	3.16	4.8
S.E. (4,3)		7.4	3.41	5.2
S.E. (3,3)		7.9	3.65	5.5

[abcd] Means in the same column with common-lettered superscripts are not statistically different ($P > .05$).

[1] Mg N/100 ml rumen fluid.

TABLE XVI

COEFFICIENTS OF DETERMINATION (R^2) FOR REGRESSION
OF RUMINAL FLUID FOAM STABILITY, EXPANSION AND
STRENGTH ON SOLUBLE NITROGEN FRACTIONS OF
RUMEN FLUID (1977-78)

| Dependent Variable | Number of Independent Variables | Independent Variables | | | R^2 Value |
		Total Soluble Nitrogen	Soluble Protein Nitrogen	Soluble NPN[a]	
Foam Stability	1		x		.147 *
	2	x		x	.172
	3	x	x	x	.230
Foam Expansion	1		x		.165 *
	2	x		x	.177
	3	x	x	x	.268 *
Foam Strength	1			x	.033
	2	x		x	.043
	3	x	x	x	.047

[a]Non-protein nitrogen.

*($p < .05$).

TABLE XVII

COEFFICIENTS OF DETERMINATION (R^2) FOR REGRESSION OF RUMINAL FLUID SOLU-
BLE NITROGEN, SOLUBLE PROTEIN NITROGEN AND SOLUBLE NON-PROTEIN NITRO-
GEN ON CHEMICAL COMPONENTS OF WHEAT FORAGE (1977-78)

Dependent Variable	Number of Independent Variables	Independent Variables						R^2 Value
		Dry Matter	NDF[a]	Crude Protein	Total Soluble Nitrogen	Soluble Protein Nitrogen	Soluble NPN[b]	
Ruminal Fluid Soluble Nitrogen	1			x				.489 *
	2			x		x		.508 *
	3	x		x			x	.554 *
	4			x	x	x	x	.646 *
	5	x	x		x	x	x	.725 *
	6	x	x	x	x	x	x	.732 *
Ruminal Fluid Soluble Protein Nitrogen	1			x				.459 *
	2			x	x			.472 *
	3	x		x			x	.510 *
	4	x	x	x			x	.530 *
	5				x	x	x	.552 *
	6	x	x	x	x	x	x	.553 *
Ruminal Fluid Soluble Non-protein Nitrogen	1			x				.415 *
	2	x		x		x		.453 *
	3			x		x		.516 *
	4			x	x	x	x	.624 *
	5	x	x		x	x	x	.734 *
	6	x	x	x	x	x	x	.766 *

[a]Neutral-detergent fiber.

[b]Non-protein nitrogen.

*(P < .05).

CHAPTER V

GENERAL DISCUSSION

These data emphasize the need of determining where major emphasis
should be placed in regard to measurements of bloat potential, and
its prediction from forage chemical components. Pressey et al. (1963b),
Miltimore et al. (1964), Stifel et al. (1968a), Rumbaugh (1969) and
Howarth et al. (1975 and 1977) attempted to associate the incidence
and/or degree of bloat with various forage chemical components.
Coefficients of determination reported between the incidence and/or
degree of bloat and various forage chemical components were, respective-
ly: R^2 = .31 (P < .01); R^2 = .29; R^2 = .72 to .85; R^2 = .03 (P < .1);
R^2 = .01 (P > .1) to .12 (P < .005). In general, the magnitude of most
of the reported R^2 values has been very low. The coefficients of
determination (.72 to .85) reported by Stifel et al. (1968a) were
between extent and strength of calcium and magnesium binding to Frac-
tion I chloroplast proteins at pH 5.5 and the incidence of bloat.
Alfalfa plants (5 from each of 20 locations) were randomly cut,
mechanically chopped and fed to the assay animals. The animals were
assigned bloat scores on a basis of 0 to 5 at frequent intervals
following feeding in order to obtain an overall score for bloat
severity. The highest score obtained for each animal during the feed-
ing period was used. The R^2 value of .31 (P < .01) reported by

Pressey et al. (1963b) was between the incidence of bloat in cattle grazing alfalfa and stability of foams generated from homogenated alfalfa extracts. The homogenates were combined with 60 ml of 0.2 M phosphate buffer, pH 6.5, with the sample container immersed in water at 39 C. Nitrogen gas was bubbled through the solution at a constant rate of 4389 cm^3 per hour for 1.75 minutes. Measurements of foam stability were based on the rate of decomposition of the foam as determined by the rate at which the liquid collected in the column. Results were recorded as the volume of liquid remaining in the form of foam. The R^2 value (R^2 = .29) reported by Miltimore et al. (1964) was between the incidence of bloat and the crude protein content of alfalfa. The alfalfa was cut shortly after 8 a.m. daily, and was fed to 14 lactating purebred Jersey cows. At each observation, each animal was scored according to the severity of bloat signs on a scale of 1 (normal animal) to 7 (animal died of bloat). Average daily bloat incidence was the sum of the highest score for each animal divided by the number of animals on test.

In addition to the varying degree of variation in the incidence and/or degree of bloat accounted for by forage chemical components, each group of researchers has defined or measured the respective parameters with different terms and apparatuses. This has further complicated interpretation of results. Furthermore, very few studies have measured foam parameters of ruminal fluid as the foaming media. These are perhaps reasons which have contributed to the general failure of identifying the specific cause(s) of bloat, since it was first described in 60 A.D. (Church, 1969).

The soluble nitrogen fractions reported in this study agree
quite well in definition and technique with those examined by Howarth
et al. (1975 and 1977). Although in these studies with wheat pasture,
a greater portion of the variation in ruminal fluid foam measurements
(indices of bloat potential) was accounted for by wheat forage
chemical components. Howarth et al. (1975 and 1977) employed incidence
of bloat to the forage chemical components of alfalfa cultivars.
Horn et al. (1976) found differences (P < .05) between wheat forage
chemical components from bloat-prococative wheat pastures and wheat
pastures where stocker bloat had not occurred. These wheat forage sam-
ples were obtained from pastures around the State of Oklahoma when bloat
occurred, and therefore ruminal fluid samples could not be collected to
examine possible relationships between ruminal fluid foam measurements
and wheat forage chemical components. Rumen fluid was used in these
studies based on the opinion that animal factors and samples from an
in vivo ruminal environment are pertinent to an understanding of the
etiology of bloat (Clarke and Reid, 1974). In analyzing the steer
parameters, no steer effects (P > .05) were observed. The largest
R^2 value obtained in this study was .501 (P < .05; Table XI).

Since forage intakes were not measured in these studies, a large
portion of the unaccounted variation could possibly be accounted for
by rate of forage intake of wheat pasture stockers. Rumen fill has
been suggested by various workers as being an important factor in
accounting for the efficiency of eructation and ruminal contractions
(Dougherty, 1940; Dougherty et al., 1958; Stevens and Sellers, 1959
and 1960). Stevens and Sellers (1960) recorded a lowering of the

contents of the rumino-reticulum of 10-15 cm and a raising of the pos-
terior dorsal sac of 10 cm in ruminally cannulated sheep that were insuf-
flated to an intraruminal pressure of 30 mm Hg in the dorsal sac. If the
cardia is covered with fluid (including froth) the reflex opening of
the cardia does not occur (Dougherty et al., 1958). Dougherty (1940)
and Stevens and Sellers (1959) further noted that if the rumen was
insufflated with gas experimentally, there was an initial increase in
the frequency of the β contractions and in eructation. In light of
these observations, along with the hypothesis of the effect of aggita-
tion in protein denaturation in the rumen (McArthur et al., 1964), the
concentration of surface-active agents and ruminal motility could both
play significant roles in the etiology of bloat.

The generally high amplitudes and frequencies of ruminal con-
tractions observed during the 1975-76 and 1976-77 wheat pasture grazing
seasons may be indicative of large forage intakes. However, amplitudes
of ruminal contractions of all 4 steers on March 1, 1977, (Table IV)
were lower ($P < .05$) than the pre- and post-wheat pasture mean. Large
intakes of wheat forage could conceivably inhibit the opening of the
cardia due to bulk-fill, and therefore effect an increase in intra-
ruminal pressure and a reduction in ruminal motility (Dougherty et al,
1958; Titchen, 1968; Akester and Titchen, 1969; Leek, 1969a). Fre-
quency of ruminal contractions measured on March 1, 1977, were not,
however, decreased by whatever factors caused a reduction in amplitude
of ruminal contractions.

Although no signs of bloat were observed in the experimental
steers during the three-year study, the possible importance of rate

of forage intake over a short period of time was further suggested

by the death, due to bloat, of a Holstein cow on December 7, 1976.

This cow died shortly after being turned out on a wheat pasture

adjacent to the wheat pasture utilized in these studies. The dairy

cattle were allowed to graze wheat pasture 3 hours per day. There

was no indication from the wheat forage chemical composition data

(Appendix Table XIX) that the forage was particularly condusive

to bloat during this time. Horn et al. (1974) observed the grazing

behavior of stocker cattle on wheat pasture and observed that wheat

pasture stockers go off feed prior to the movement of weather fronts

through an area and then consume large amounts of forage after the

passage of the weather fronts. About 25% of the calves exhibited

ruminal distension and were believed to be bloated. An increased

forage intake might alter the rumen system sufficiently to induce bloat.

According to Hancock (1954) the rate of intake is not important,

although Mendel and Boda (1961) reported a higher dry matter content

in boluses from bloat susceptible cows. Reid et al. (1972) attributed

this difference in dry matter content to be partly related to feed

intake.

At present, the results of studies which have attempted to

identify the etiological factors of bloat and their relative importance

are inconclusive. In order to obtain a more integrated picture,

standard criteria must be established on which to base results and

therefore contribute meaningful data to the etiology of bloat. This

author suggests a more adequate study of the relationships concerning

an in vivo ruminal environment.

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APPENDIXES

TABLE XVIII

WHEAT FORAGE COMPOSITION[1], 1975-76

Date	Dry Matter %	Crude Protein %	Soluble Carbohydrates %	Foam-Stabilizing[2] Protein	Foam-Stabilizing[3] Protein
11-23-75	27.85^c	22.16^g	17.12^i	469.5^a	3.6^a
12-01-75	28.91^{cd}	19.96^{ef}	18.23^{ij}	831.4^a	6.9^a
12-09-75	31.49^{ef}	20.09^{ef}	15.64^h	899.0^a	6.9^a
12-16-75	33.10^f	20.06^{ef}	15.53^h	534.2^a	5.0^a
12-23-75	18.60^{ab}	20.40^{ef}	12.03^{fg}	980.4^{ab}	12.3^b
12-30-75	17.68^a	20.94^{fg}	9.34^{cd}	954.2^{ab}	14.0^{bc}
1-06-76	40.65^h	19.13^{de}	14.77^h	1427.9^c	13.3^b
1-15-76	50.42^j	16.75^{ab}	15.20^h	2427.2^{fg}	34.0^{fg}
1-22-76	50.41^j	16.40^{ab}	14.77^h	1943.8^{de}	25.9^d
1-29-76	50.82^j	17.00^{abc}	14.91^h	1382.0^{bc}	15.8^{bc}
2-05-76	45.99^i	17.29^{abc}	12.64^g	1466.7^c	17.2^{bc}
2-12-76	42.70^h	16.03^a	10.33^{de}	2081.9^{ef}	29.3^{ef}
2-19-76	36.44^g	21.00^{fg}	8.07^{ab}	2608.5^g	32.7^{efg}
2-26-76	42.42^h	17.60^{bc}	11.91^{fg}	2332.6^{efg}	27.0^d
3-05-76	30.59^{de}	16.45^{ab}	8.22^{abc}	2162.8^{efg}	28.7^{de}
3-11-76	29.92^{cde}	20.19^{ef}	8.50^{bc}	1484.1^{cd}	18.4^c
3-17-76	24.35^b	24.37^h	11.36^{ef}	5225.4^j	47.9^i
3-25-76	22.17^b	24.80^h	7.31^a	3258.2^h	35.8^{gh}
4-01-76	29.77^{cde}	18.40^{cd}	19.77^{ij}	3949.1^i	39.3^h
S.E.	1.5	.89	1.13	446.9	4.9

[1] Mean values of 6 forage samples.
[2] Foam-stabilizing protein (units x 10^{-4}/gm forage dry matter).
[3] Foam-stabilizing protein (units x 10^{-4}/mg protein).
abcdefghij Means in the same column with common-lettered superscripts are not statistically different (P > .05).

TABLE XIX

WHEAT FORAGE COMPOSITION[1], 1976-77

Date	Dry Matter %	Neutral-Detergent Fiber	Crude Protein %	Total Soluble Nitrogen[2]	Soluble Protein Nitrogen[2]	Soluble Non-Protein Nitrogen[2]
11-04-76	22.65^a	46.30^a	28.82^i	1.84^a	$.68^e$	1.16^c
11-11-76	21.78^a		25.69^{fg}			
11-18-76	31.77^{de}	60.55^{cd}	25.44^{fg}	1.34^a	$.24^a$	1.10^{bc}
11-26-76	32.71^{def}		26.12^g			
12-02-76	31.96^{def}	56.17^{bc}	24.98^{efg}	1.33^a	$.41^{abcd}$	$.92^a$
12-09-76	33.84^{fg}		25.96^{fg}			
12-16-76	33.73^f	59.83^{cd}	26.11^g	2.21^c	$.49^{cd}$	1.72^e
12-21-76	32.91^{def}		26.35^{gh}			
12-28-76	33.62^{ef}	56.35^{bc}	26.08^g	2.26^c	$.57^{de}$	1.69^e
1-04-77	37.58^h		22.70^{ab}			
1-20-77	37.35^h	61.00^d	23.49^{bcde}	1.82^b	$.35^{abc}$	1.47^d
2-01-77	35.99^h	60.96^d	21.18^a	1.83^b	$.47^{bcd}$	1.36^d
2-08-77	35.78^{gh}		23.29^{bcd}			
2-15-77	25.91^b	59.60^{cd}	28.91^i	1.42^a	$.28^{ab}$	1.14^{bc}
2-22-77	27.02^{bc}		27.86^{hi}			
3-01-77	25.80^b	52.47^b	28.44^i	2.35^c	$.56^{de}$	1.79^e
3-08-77	32.07^{def}		23.58^{bcde}			
3-15-77	28.58^c	47.73^a	24.44^{cdef}	1.80^b	$.82^f$	$.98^{ab}$
3-22-77	31.57^d		22.97^{bc}			
S.E.	1.18	2.77	.95	.13	.10	.10

[1]Mean values of 6 forage samples.

[2]Percent dry matter.

[abcdefghi]Means in the same column with common-lettered superscripts are not statistically different

TABLE XX

WHEAT FORAGE COMPOSITION[1], 1977-78

Date	Dry Matter %	Neutral-Detergent Fiber %	Crude Protein %	Total Soluble Nitrogen[2]	Soluble Protein Nitrogen[2]	Soluble Non-protein Nitrogen
11-11-77	21.70^b	48.89^b	22.88^b	1.58^c	$.22^a$	1.36^f
11-22-77	26.67^c	49.40^{bc}	20.82^a	1.39^b	$.21^a$	1.18^e
12-09-77	34.15^d	49.42^{bc}	19.95^a	1.19^a	$.26^{ab}$	$.93^{bc}$
12-21-77	36.35^d	49.77^{bc}	20.29^a	1.24^a	$.38^{cd}$	$.86^{ab}$
1-06-78	40.97^e	53.60^d	21.15^a	1.14^a	$.31^{bc}$	$.83^a$
3-09-78	41.96^e	52.01^{cd}	20.75^a	1.37^b	$.49^f$	$.88^{ab}$
3-21-78	28.08^c	49.33^{bc}	27.99^c	1.43^b	$.40^{de}$	1.03^d
4-06-78	18.65^a	45.10^a	29.59^d	1.45^b	$.45^{ef}$	1.00^{cd}
S.E.	1.28	1.70	.86	.06	.04	.05

[1]Mean values of 4 forage samples.

[2]Percent dry matter.

[abcdef]Means in the same column with common-lettered superscripts are not statistically different (P>.05).

TABLE XXI

ANALYSES OF VARIANCE FOR RUMINAL FLUID FOAM STABILITY,
EXPANSION AND STRENGTH , 1977-78

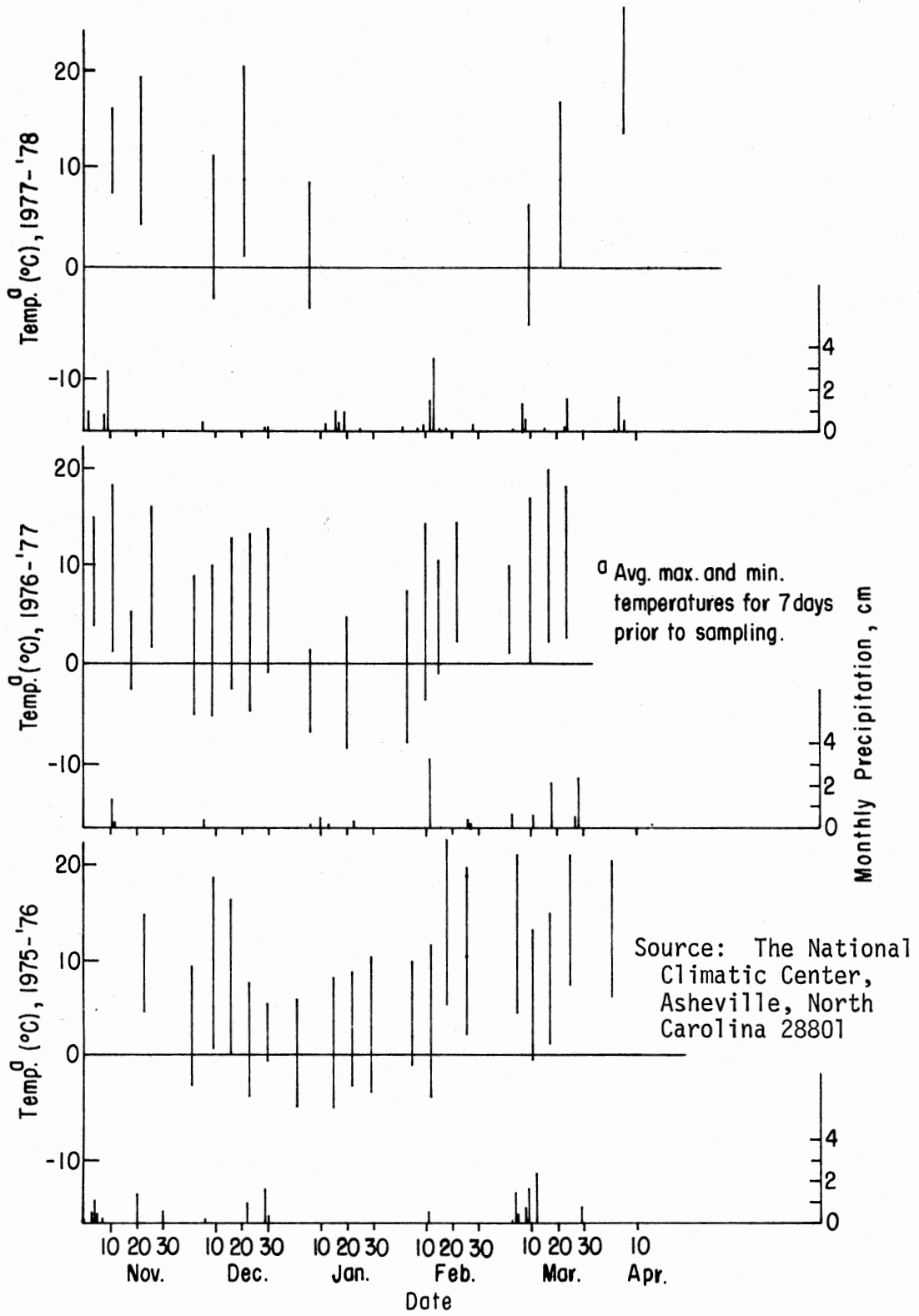|	Source of Variation	df	Sum of Squares	Mean Square	F	P > F
Foam Stability	total	28	14.39	---	---	---
	steer	3	.20	.06	.13	.94
	date	7	5.11	.73	1.45	.24
	error	18	9.08	.50	---	---
Foam Expansion	total	28	14.11	---	----	---
	steer	3	1.08	.36	.73	.54
	date	7	4.28	.61	1.24	.32
	error	18	8.75	.49	----	---
Foam Strength	total	28	262.04	---	----	---
	steer	3	23.42	7.81	1.03	.40
	date	7	102.11	14.59	1.92	.12
	error	18	136.51	7.58	----	---

Figure 12.   Monthly Precipitation and Average Minimum
             and Maximum Temperatures, 1975-76,
             1976-77 and 1977-78

VITA

Denzil Fay Frost

Candidate for the Degree of

Master of Science

Thesis: RUMINAL MOTILITY AND BLOAT POTENTIAL OF WHEAT PASTURE STOCKERS

Major Field: Animal Science

Biographical:

Personal Data: Born in Kanab, Utah, November 11, 1950, the son
of Mr. and Mrs. Denzil M. Frost and married Paula Jean Smith,
L. D. S. St. George Temple, September 9, 1972.

Education: Graduated from Kanab High School, Kanab, Utah, in May,
1969. Received the Bachelor of Science degree in Agriculture
from Purdue University in West Lafayette, Indiana, in Decem-
ber, 1975, with a major in Animal Science. Completed the
requirements for the Master of Science degree from Oklahoma
State University, Stillwater, Oklahoma, in July, 1978.

Professional Experience: Ranch worker and laboratory technician
at Purdue University. Graduate Assistant at Oklahoma State
University, 1976-1978.

Professional Organizations: Member of American Society of Animal
Science, Alpha Zeta and Sigma Gamma Delta.