FERMENTATION PRODUCTS AND DIGESTIBILITY OF ALFALFA, CENTER-CUT CORN SUDAN AND SUDAX SILAGES TREATED WITH THREE DIFFERENT ADDITIVES

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Joseph A. Atekwana

1965

Fermentation Products and Digestibility of Alfalfa, Center-cut Corn, Sudan and Sudax Silages Treated with Three Different Additives

Fermentation Products and Digestibility of Alfalfa, Center-cut Corn, Sudan and Sudax Silages Treated with Three Different Additives

by

Joseph A. Atekwana

AN ABSTRACT OF A THESIS

Submitted to
Michigan State University
in partial fulfilment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Animal Husbandry

1965

ABSTRACT

Additive effect on the fermentation products and dry matter digestibility of silages was studied. Forages ensiled in miniature metal silos under 10 lb. per square inch pressure were alfalfa, center-cut corn, sudan grass and sudax. Urea (42%N) and two commercial silage additives, were used.

The experiment was a factorial design with four treatments on four forages and one replicate.

The forages from pre- and post-fermentation were analyzed for dry matter, pH, crude protein, volatile fatty and lactic acids.

Digestion trials, using the modified nylon bag technique, were carried out by incubating the silages in the rumen of a fistulated Holstein steer.

Silages treated with urea resulted in significantly ($P < .05$) higher protein, pH, acetic and lactic acid contents. The effects of the other additives were erratic.

Unfermented forages had significantly ($P < .05$) higher percent apparent dry matter digestion than the fermented ones. There was an increase in dry matter digestion with increased time in the rumen. The highest dry matter digestion occurred between the 24 and 36 hour periods.

Correlation coefficients of 0.91 and 0.84 were observed between pre-and post-fermentation dry matter content and between crude protein and 24 hour period digestion respectively. There was a negative correlation (coeff. -0.58) between the 12 hour post fermentation digestion period and lactic acid content.

Fermentation Products and Digestibility of Alfalfa, Center-cut Corn, Sudan and Sudax Silages Treated with Three Different Additives

by

Joseph A. Atekwana

A THESIS

Submitted to
Michigan State University
in partial fulfilment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Animal Husbandry

1965

ACKNOWLEDGEMENTS

The author wishes to express his unqualified appreciation to Dr. H.W. Newland, Associate Professor, Department of Animal Husbandry, for his help in planning the study, and his invaluable time spent correcting the manuscript.

The author also expresses deep appreciation to Mr. B.E. Brent, Chemist, Animal Husbandry Nutrition Laboratory, without whose help chemical analyses and calculation of the results would have been difficult.

Thanks is also due the Staff of the Dairy Science Department Laboratory for allowing the author the use of their facilities including the gas chromatograph in the volatile fatty acid determination. The same thanks are also extended to Dr. S.T. Dexter, Professor Crop Science Department for permitting the use of his miniature metal silos for the silage fermentation. The author is most grateful to Dr. D.E. Ullrey, Associate Professor, Animal Husbandry Department and Dr. R.W. Luecke, Professor, Biochemistry Department; members of his academic committee, for proofreading and suggesting corrections in the manuscript. Special thanks are here extended to Mr. Arikpo E. Ettah, whose companionship created a continuous congenial atmosphere in which to work. It would be impossible to name all other friends who lent the author a helping hand during the study. To these sincere friends the author is deeply grateful.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued) .....

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

The problem of equitable distribution of animal feed through the various seasons of the year is one with which livestock farmers are familiar. Sometimes, farmers in the temperate regions of the world have more forage than they can profitably handle in early summer and in winter; their fields are completely covered with snow. The same is true of farmers in the tropics where there are two distinct seasons - the dry and wet seasons. During the wet season, there is an abundant supply of forage and in the dry season, the fields are virtually scorched by the sun.

Silage making has become one of the best weapons of the farmers against these seasonal irregularities in livestock feed supply. The farmer has also realized that in cutting, hauling, ensiling and storing forage, he loses some of the vital nutrients. Consequently, he has tried many ways to salvage this nutrient loss during silage making. One of the well known ways he has done this is to treat the silage with various additives. Some of the commonly used additives have been mineral acids like sulfuric, hydrochloric and phosphoric. Others like urea, calcium carbonate and yeasts have also been used with varying success. Some farmers have also used microorganisms for good silage production.

In using these additives, the silage makers have often asked the question of how much and what kinds of nutrients are lost during the ensiling process. They also want to know how much of these nutrients the additives can save the most. Various researchers have addressed themselves to some of these pertinent questions and have come up with answers which are by no means conclusive. It was, therefore, in the search of further answers to these questions that this research has been conceived. The data which are to be presented in this manuscript deal with the effects of some of these additives on the chemical processes and transformations occurring in the ensiled products. Two rather recent commercial additives - Bio-zyme referred to as "P" and Silo Guard referred to as "Q" - have been used in addition to urea. The ensiled materials treated with these additives were alfalfa, center-cut corn, sudan grass and sudax - A cross between sorghum and sudan grass.

It would have been necessary to carry out some feeding trials with livestock to find out the effect of additive treatment on the metabolism of these silages. But this was not possible because the quantities of the silages made in miniature silos were inadequate. However, some digestibility - dry matter disappearance - trials were carried out using the modified nylon bag technique in the rumen of a fistulated steer. These data too, will be presented.

The researcher also believes that grass silage making will become an important operation in livestock production,

especially among the developing nations of the world where grasses abound, concentrates are in short supply and the competition is acute between man and beast for food.

REVIEW OF LITERATURE

Roughage occupies a unique place in the nutrition of ruminants. Among the various forms of roughage that comprise livestock rations, silage is of prime importance. It is then no wonder that the production of silage has received considerable attention since man began the domestication of animals.

1. Silage Fermentation

Some of the earliest work done on silage is recorded in Food & Life, the Year Book of Agriculture, 1939. It is stated in this publication that silage production as we know it today was started by a Frenchman named Goffart in 1875. Woll, the author of the article stated that the idea of modern silage was brought to the United States by Morris in Maryland in 1876.

Watson *et al.* (1937) and Woll (1939) both maintained that livestock farmers should prefer silage over hay because in harvesting and storing forages or hay they lose about 20 percent of the nutrients (including total loss by fire) as compared to almost negligible losses as silage. Allen *et al.* (1937) observed that the rapid changes taking place in the ensiled mass were influenced by (a) external temperatures, (b) moisture content, (c) botanical species, (d) age or

stage of maturity of the silage material and also (e) by the season during which the silage crop was harvested. They also said that the compaction of silage during filling of the silo to exclude as much air as possible enhanced silage quality. Allen et al. also observed that the main chemical changes in the silage took place within the first ten days. This point is supported by numerous other investigators (Russell, 1908; Watson, 1937; Virtanen, 1938 and Barnett, 1954).

Writing about the sequence of changes taking place in the ensiled material, Russell (1908) reported that the general chemical changes known to occur in silage were the conversion of sugars to carbon dioxide and water, the production of acetic, butyric and lactic acids and the production of non-protein material from proteins by the process of deamination.

I. Acid Production

Most of the investigations into the changes that silage material undergoes have centered around the chemistry associated with production of volatile and non-volatile fatty acids and the period of most rapid fermentation. Esten and Mason (1912) concluded that the most important period of corn silage fermentation began shortly after the material was ensiled and was usually completed within a few days. Allen, et al. (1937) studied the chemical and bacteriological changes occurring in grass silage, and

reported that most changes were initiated by the microflora that was usually carried on the fresh crop. He observed that the coliform bacteria and some rods, mostly lactobacilli, appeared to be prevalent in the fresh material and hence in the silage. He went on to report that the coliforms were of a type that would not thrive above 30°C. In this case, the coliforms were the dominant bacteria in the early days of silage fermentation before the temperature rose above 30°C and from then on, lactobacilli which could thrive at 37°C and above became prevalent and multiplied rapidly just after the decline of the coliforms.

The progress of fermentation is associated with higher temperatures and lower pH. Since a rapid increase in both temperature (to a certain point) and pH will inhibit the growth of the microorganisms that will produce undesirable acids like butyric, the earlier these conditions are achieved, the better the silage quality. It is, therefore, important that the acidity be rapidly increased. "The pH of the silage is one of the best indices of its value" (Watson, 1937). Low pH can be achieved by directly adding acids to the silage or adding fermentable carbohydrates which lactobacilli will use in lactic acid production. Thus, acid and molasses addition at the time of ensiling will produce an excellent product if care is taken to achieve great compaction. Ferguson & Watson (1937) observed that when the pH did not exceed 4.5, the quantity of butyric acid, an indication of putrefaction, was negligible. They

also reported that acetic acid was the main constituent of the volatile fatty acids while lactic comprised most of the non-volatile fatty acids. However, they noted that in cases where butyric acid exceeded 0.75 to 1 percent by weight of the fresh silage, care had to be taken to feed the silage outdoors or in the open air. Thus, according to Ferguson & Watson, the aim for good silage was to produce a minimum of butyric acid. This observation was shared by many other investigators including Hayden et al. (1945), Herman et al. (1941) and Virtanen (1933) as quoted by Watson (1939).

Virtanen (1938) reported that Edin and Sandberg (1922) were the first to determine the pH in silages. Virtanen (1938) was stimulated by Edin & Sandberg's work to start a systematic investigation of the processes taking place in silage and their effects on silage quality. Among the detrimental processes, he cited protein breakdown and the fermentation caused by the coliform and the butyric acid bacilli. Irwin (1956) working with orchardgrass silage, Langston (1958) and Kempton and San Clemente (1959) working with thirteen different grass silages reported on their findings which were in agreement with those of Virtanen (1938). Barnett (1954) reported that the process of ensiling meadow and corn crops was characterized by the fermentation of carbonhydrates which resulted primarily in lactic and acetic acids. In their studies of the use of urea to increase the crude protein content of corn silage for fattening steer,

Bentley et al. (1955) reported that organic acids, especially acetic and lactic, had a high feed replacement value and that production of these acids should be encouraged whenever a silage fermentation scheme was planned. This view was also expressed by Dobrogosz and Stone (1957) when they used metabisulfite to increase acid production in alfalfa silage. In this same study 0, 8 and 12 pounds of metabisulfite were used per ton of silage, and the authors concluded that the utilization of sugars and the production of acids in the silages was inversely correlated with the amount of metabisulfite added to the silage.

Many other workers have continued to investigate acid production in silages. Klosterman et al. (1960) analyzed the organic acids produced in whole corn plants ensiled in large glass jars and treated with various neutralizing agents. These neutralizers consisted of (1) 0.5 percent low magnesium limestone plus 0.5 percent urea, (2) 0.5 percent urea, and (3) 1.0 percent urea. The acidity of these silages as determined by the pH was 4.30, 4.10, and 4.40, respectively. The acetic acid content of the three silages was 2.13, 1.93, and 1.71 percent on dry matter basis, respectively. The lactic acid content was 12.05, 8.71 and 12.00 percent, respectively.

Klosterman et al. (1962) treated ear corn silage with different additives and increased the moisture content by adding water. They reported that the difference in acidity between the treatments, as measured by the pH, was

not statistically significant but that there was an increase in lactic and acetic acid production. A report by Hall *et al.* (1954), in which sweet potato vine silage was treated with additives showed that the silage had an "acid type" fermentation with good odor, color and texture. Lactic acid formation increased four fold in the first four days due to the increase of lactobacilli *Plantarium*. The chemical changes were evidenced by a rapid drop in the pH and a rise in lactic acid production. Acidity was further increased by the addition of tubers and molasses to the vines.

One of the most detailed studies on acid production in silage was done by Irwin *et al.* (1956). They determined formic, acetic, proprionic, butyric, succinic and lactic acids during the 40th to 60th days of silage fermentation. The experiment was designed in such a way that poor to good quality silages were produced. Prior to ensilation, acid content on a dry matter basis was less than 1 percent of the fresh grasses. Silages classed as "mediocre" had butyric acid after five to eight days. Lactic acid increased up to 10 percent on a dry matter basis within the first five days then stabilized. Proprionic and succinic acids were negligible.

II. Heat Production

Most of the chemical reactions taking place within the ensiled mass is associated with heat production. This is governed by the general environment and such internal

factors as degree of aeration and wetness of the ensiled mass. Temperatures in excess of 100°F were associated with scorched silage with excessive dry matter loss, and temperatures below 75°F were sometimes associated with poor quality silage, characterized by butyric acid production according to Briggs et al. (1959). A report by Benne and Wacasey (1960) closely agreed with Briggs findings. They showed that temperatures between 80°F to 100°F produced cold and those from 100°F to 120°F produced warm fermentation. This would seem to conflict with Benne and Wacasey (1960) who considered 80°-100°F favorable to the production of good silage.

These findings have stressed the effects of both excessive and suboptimal temperatures on silages. At the Imperial Chemical Institute Experiment Station at Lealott's Hill, England, Watson et al. studied "losses of dry matter and digestible nutrients in low temperature silage with and without added molasses or mineral acids."

They found that under the low temperature conditions dry matter loss in five silages ranged from 13.7 to 31.1 percent. Seepage was also blamed for most of the dry matter loss. Watson et al. (1937) observed that about 38.7 percent of the bases and 85 percent of volatile fatty acids were lost during drying. One of the nutrients affected most by temperatures is digestible crude protein. Bratzler et al. (1956) remarked that under high temperature conditions, "a silage may lose as much as 30 percent of its original crude

protein and still contain a higher percentage of this nutrient at the end of the storage period due to large carbohydrate losses." Watson et al. (1930) also found that higher temperatures in the silage rendered proteins indigestible. Others who reported studies on silage temperatures are: Hunter (1917) who measured a temperature of 30°-40°C in the center of the silo; Allen (1937) who measured 90°F and 102°F for the top and bottom of the silo, respectively.

Sherman and Bechdel (1918) found that corn stover silage containing excess water had a temperature of 57.7°F. On the other hand Kempton and San Clemente (1959) reported temperatures of 111°F and 131°F in one case and 114°F and 130°F in another in silages with excess water. These temperature readings were taken from the top and bottom of the silos and the highest ones were considered detrimental to good silage material.

III. Additives

It was noted in the introduction that one of the best ways farmers have achieved desirable silage quality has been through the use of appropriate additives. Urea, calcium carbonate, calcium phosphate, whey, molasses and mineral acids have been some of the additives used. (a) Urea: The use of urea as a protein substitute in the ration of ruminants has assumed greater importance with the increased use of concentrates. One way of getting urea into animal feed has been through its addition to silages at the time of ensilation.

Woodward and Shepherd (1944) ensiled corn silage using 10 pounds of urea per ton. They later fed some of the treated silage to milk cows with a low protein concentrate ration. Another group was fed urea to boost the protein intake, but this time the urea was added directly to the concentrate. No significant differences were noted between treatments, and milk production remained on a high plane. This showed that whether urea was incorporated into the silage or fed along with concentrates, the net effect remained the same. It was further learned that additional increases in urea had deleterious effect on the palatability of either the silage or the concentrate.

In 1944, Davis et al. used a urea solution in quantities of 0, 10, 30, and 50 pounds of urea per ton of sorghum silage. Crude protein determinations prior to and after fermentation showed that there was a slight migration in nitrogen and that free ammonia was noticed from the silages with higher concentrations of urea. There was a great variation in pH. The control silage had pH 3.5 while the silage with 50 pounds of urea per ton had a pH of 7.6. The cattle that were fed these silages showed no discernible difference between the 0 and 30 pound urea silages but completely refused to eat the 50 pounds per ton urea silages. However, when free ammonia had been released they ate this silage. Cullison (1944) quoted Harris and Mitchell (1941) as having improved the digestibility of a low protein ration for sheep by supplementing with urea. The improvement

was explained as due to the stimulation of microflora in the rumen which enhanced carbohydrate fermentation. With the addition of urea to the silage, prolonged fermentation stopped and the resulting silage was superior to that which was ordinarily made in carotene content, palatability and general feeding value for lambs and beef cattle.

The use of urea as an additive to silage continued to be investigated by other workers. Means (1945) compared urea treated and untreated sorghum silages. He used silage to which 10 pounds per ton urea was added. He carried out feeding trials for a 77 day period. Three lots of beef cows and three lots of yearling heifers were used. The rations were: (1) A standard ration of 30 pounds of untreated sorghum silage, 1 pound of cottonseed meal and 5 pounds of Johnson grass hay; (2) 35 pounds of the same untreated silages, 5 pounds of Johnson grass hay; (3) 35 pounds of urea treated silages, and 5 pounds of the same hay. The heifers were fed 5 pounds less than the cows in a combination of these ingredients. The cows in Lots 1, 2, and 3 gained 9 pounds, lost 99 pounds and gained 13 pounds respectively. Heifer calves, differed although the standard ration produced the best gain, still the treated ration remained superior in an overall consideration. In studying the effect of urea-treated and untreated corn silage in the performance of lactating dairy cattle, Wise et al. (1944) noticed that the treated corn silage had carmalized odor and brownish color. In terms of dry matter, it took 16.9 pounds and 15.5 pounds

per animal daily of untreated and treated silages, respectively. Although the results of Wise *et al.* seem to differ from those of Means (1944) the work of Cullison (1944) agreed with Means. Feeding results obtained by Wise agreed with those of Woodward and Shepherd (1944). One important point about Wise's work is that the urea-treated silage had a crude protein content of 10.79% as compared to 7.48% for the untreated silages. Similar results were reported by Bentley *et al.* (1955). In the same work, Bentley reported that urea-treated corn silage was not inferior in feeding value to corn and soybean oil meals. Contrary to this finding, Archibald and Parsons (1945) found urea-treated silage quite unsatisfactory because of the conversion of the urea to ammonia with objectionable odor. Hall *et al.* (1954) found the color, odor and texture of sweet potato vine silage treated with urea to be good. One of the most recent studies, in the investigation of the addition of urea to silage, was done by Klosterman *et al.* (1960) and (1961). In one of these studies, they found that 0.5 percent urea treated silage increased the lactic acid production 75 percent over that which was not treated; on a dry matter basis. However, they also noticed a slight loss in dry matter in the treated silage. This brief review of the value of adding urea to silages and to livestock feed has shown a few outstanding things. These are: that urea has a "protein sparing" function. It arrests delayed fermentation and accentuates the production of lactic acid. Animals fed urea

treated silages have generally shown consistent gains and thrift in feed conversion.

(b) Mineral Acids: The principal inorganic acids used as silage additives and preservatives have been hydrochloric, sulfuric and phosphoric. Virtanen (1933) as quoted by Watson (1939) was one of the strong preponents for silage treatment with mineral acids. He carried out intensive experiments in which he used hydrochloric, phosphoric and lactic acids to depress the pH of silages to 3.6. In this work hydrochloric acid was superior to the other two in depressing pH to the desirable range. Other workers, Stone et al. (1943) observed that phospharic acid applied at 16 pounds per ton of silage depressed the pH and resulted in good silage product if care were taken to include fermentable sugars with the ensilage. A classic example of the use of phosphoric acid in silage work was reported by Ingham et al. (1949). In five years of intensive research in silage fermentation, with molasses and phospharic acid, they arrived at some interesting conclusions. The silages produced in each of five experiments were fed to dairy cattle and records of milk production, physiological effects on the animals and palatability were kept. The design of experiments was basically the same. In the first trial, one lot of three Holsteins and two Guernseys were fed corn silage, mixed hay and a grain mixture. A second lot of the same number and types of dairy cattle was fed molasses - alfalfa silage, mixed hay and a grain mixture. The third

group of the same number of animals was fed phosphoric acid - alfalfa silage, mixed hay and the same grain mixture. The fourth group had only molasses - alfalfa silage alone and the last group was fed phosphoric acid - alfalfa silage alone.

In general the results showed that cows on alfalfa silage had a positive nitrogen balance. Exceptions were observed in a 28 day trial in which limestone was added to the phosphoric acid treated silage of lot five. In this period, cows in lot five showed a negative nitrogen balance of 13 gm. daily. However, for every pound of digestible nutrient consumed, lot five which was fed the phosphoric acid silage produced 1.63 pounds of milk as compared to 1.42 pounds for lot four fed the molasses silage. The researchers explained this by saying that the cows in lot five seemed to have been inherent good producers.

In one of the experiments in which oats substituted for alfalfa, the phosphoric acid - oat silage promoted greater protein digestibility. In conclusion, the authors noted that the decisions as to which additive to use in a silage should be made with the local conditions in mind. However they maintained that "silage preserved with phosphoric acid definitely hinders, strange as it may seem, the retention of phosphorus."

Allen et al. (1937) observed that addition of mineral acids reduced protein breakdown. Similar observations were made by Herman et al. (1941). They dug out two trench silos with capacities to hold 45-50 tons. These silos

were 8 feet wide at the bottom and 12 feet at the top. In one of the trench silos, barley silage was ensiled with 60 pounds of molasses per ton. The second had eight pounds of 75 percent phosphoric acid per ton. Except for peripheral spoilage, both silages did not have any discernible differences either in color, odor, or texture. Work reported by Colovas et al. (1958) showed that calcium phosphate depressed the digestibility of the silages due to the release of phosphorus during decomposition.

(c) Calcium Phosphate and Calcium Carbonate: The addition of calcium phosphate and limestone to silages is a relatively recent concept. One of its early investigators is Colovas et al. (1958) who used twelve dairy heifers to determine the effect of pulverized limestone and calcium phosphate on the nutritive value of dairy feeds. They fed ladino clover, bromegrass, timothy and grass-legume silages with limestone and calcium phosphate at the rates of 50 and 100 grams per head daily in the silages. During the second year of the same experiment, the ration varied slightly. They included 16 pounds of crude protein concentrate mixture. The feeding of 100 grams of pulverized limestone daily depressed the crude protein digestibility and energy of the silages. On the other hand, 50 grams did not depress the above nutrient digestibility to any appreciable extent. They noted that two percent of dicalcium phosphate did not depress the same nutrients while the addition of one percent of limestone depressed the digestibility of both the protein and energy.

The authors then fed 2 percent calcium phosphate and 2 percent limestone and found that the depressive effect of limestone on crude protein was minimized. They concluded that limestone depressed while phosphorous enhanced the digestibilities of protein and energy of silages.

Klosterman et al. (1961) at the Ohio Agriculture Experiment Station reported the use of limestone in the treatment of high moisture corn silages. They used 0.5 percent high calcium limestone and 0.5 percent urea. In another instance, they varied the procedure, 1.0 percent high-calcium limestone was used with 60 percent water. In both trials cattle gained faster and more efficiently with the treated as opposed to the untreated silages. The production of lactic acid was significantly greater in the treated silages as compared with the untreated one or the control. There was an increase of 78 percent lactic acid in the treated over untreated corn silage and as much as 125 percent in the high-moisture calcium-treated silages. Previously Klosterman et al. (1960) reported a 100 percent increase in lactic acid in silages treated with 1 percent calcium carbonate and another increase in lactic acid of 40 percent in silages treated with dolomitic limestone over that produced by the control.

Watson (1939) also cited Sani (1912) as having treated fodder with monocalciumphosphate at the rate of 6.75 pounds per ton. The color of the treated silage remained fairly green and although odor esters were emitted

from the silage it still retained a high protein digestibility.

One of the latest investigations on the effect of calcium on silage quality was done by Nicholson et al. (1964). Nicholson noted that Byer et al. (1963) and Klosterman et al. (1962) found that the feeding value of the calcium-treated silages was higher than untreated silages, but Nicholson's group could not confirm these observations. Thomas et al. (1951) also found that treatment with limestone did not improve the feeding value of silages appreciably.

The experiment reported by Nicholson (1964) consisted of three trials. In the first trial, he ensiled the grasses in 16 oz. glass jars equipped with pressure relief valves. The grass was chopped into 1/2 to 1/4 inch lengths with a hand scissors. The material was left to ferment for 7 weeks at controlled temperature of 70°F. The silage juice was expressed and the organic acids were determined according to methods of Wiseman and Irwin (1957).

The second trial consisted of four silages fed to growing heifers in a 76 day feeding trial. Slightly wilted grass-legume hay and corn harvested at the milk stage were used. In two similar tower silos, a plastic sheet was used to divide the silos into two vertical sections. Alternate loads of 1.0 percent limestone treated and untreated materials were filled into the silos. They used 12 Holsteins, 8 Shorthorns and 4 Jerseys as test animals. Animals were

assigned to each of the four silage groups and from each class and age groups at random. Each animal received 2 lb. supplement ration in addition to water, bone meal and salt which were fed ad libitum. The third experiment compared the feeding values of six silages. Limestone at 1.0 percent and 2 percent was used in the silage each of which was made in the plastic divided silos. Two other silages of timothy in the bloom stage were made in temporary silos and these had shredded newspapers added at the rate of 5 percent to increase dry matter content. These two silages were made in silos of plastic sheets encased in snow fences. Thirty-six steers of average 655 lbs. were assigned at random to the six silages.

The digestibility trials were conducted by feeding the silages to wether lambs as the only roughage. These lambs were confined to metabolism cages. Feces and urine were collected for analysis.

At the end of these experiments, the authors concluded that addition of limestone at the time of ensiling grass or immature corn silage resulted in an increase of organic acid production during fermentation, but reduced the feeding value of the silage. They also reported that the addition of limestone to silage usually resulted in lower lactic but appreciably higher proportions of acetic and butyric acids. Feeding the limestone treated silages invariably resulted in lower feed intakes and reduced gains. The limestone did not seem to have any effect on the organic

matter digestibility of grasses but tended to reduce the digestibility of nitrogen and ash. Finally, steers fed paper-supplemented silage consumed less feed than those fed a control silage and lost weight when this was given as the only feed.

(d) Molasses and Miscellaneous Additives: As reviewed in the section of acid production in silage fermentation it seems clear that lactic acid is highly desirable. This is because of its high nutritive value. Many researchers have shown that some form of fermentable carbohydrate is desirable for lactic acid production. In most cases in which farmers have desired to increase the lactic acid content of their silages molasses has been their first choice.

Watson (1937) reported that the addition of about 0.75 to 1.0 percent of molasses in a ton of silage was sufficient to achieve the desired level of organic acid production. Hayden et al. (1945), found that molasses depressed the pH of the silages just as much as phosphoric acid. Perhaps one of the most intensive silage studies involving molasses as an additive was carried out through a five year experiment by Ingham et al. (1949).

Several silages were studied and all were compared with plain corn as the control. These silages were (1) molasses-alfalfa and phosphoric acid - alfalfa (1940-41): (2) Molasses-oat and phosphoric acid-oat 1941-42; (3) Molasses-soybean and cornmeal - soybean 1942-43; (4) Molasses - grass and ground barley grass 1943-44; and

(5) Molasses - timothy and ground barley - timothy 1944-45. In evaluating the silages the following factors were considered:

1. Feeding value - maintenance or lactation
2. Physiological effects
3. Apparent digestibility
4. Protein, calcium and phosphorus metabolism

In discussing the results, the authors observed that the feeding trials did not show any statistically significant difference among the quantities of the silage in the three balanced rations. However, the molasses-alfalfa silage was more palatable and more economical (in terms of total digestible nutrients required per pound of milk produced) than the phosphoric acid - alfalfa silage; but both were more economical than the control corn silage. There were no physiological abnormalities in the blood and urine of the cattle fed molasses-alfalfa or corn silages while in one of the lots, (phosphoric acid-alfalfa) cows had marked increases in urinary ammonia and acidity. Whereas phosphoric acid-alfalfa silages held a slight edge in crude protein digestibility over the molasses silage, the latter was superior with respect to fiber, fats and nitrogen free extracts. They found that phosphorus consumption by cows on phosphoric acid-alfalfa silage was matched by a high calcium intake. Yet, for both calcium and phosphorus, the cows had negative balances in three out of four trials whereas cows on molasses-alfalfa silage retained both calcium and phosphorus

very well. When palatability was considered in the third trial, in which molasses-soybean and cornmeal-soybean silages were used, it was found that cornmeal-soybean silage was well preserved and palatable. Plain corn silage was rated second and molasses silage was rated lowest.

In the fourth trial, the feeding value of the barley-grass silage was significantly greater than that of the molasses grass silage. Both silages were considered palatable but the animals relished the molasses-grass silage more. In the fifth and final experiment, the authors noticed that timothy silages were less palatable than the standard corn silage but the molasses-timothy silage was just as economical as corn in maintaining weight and lactation.

In conclusion Ingham et al. stated that, in general, molasses-preserved grass silages had very good palatability. Also cattle fed grass silage preserved with molasses or ground grain had excellent retention of both calcium and phosphorus. On the other hand, silages preserved with phosphoric acid definitely hindered, strange as it might seem, the retention of phosphorus.

A few non-acidic additives apart from urea, calcium carbonate and molasses have also been used in silage preservation. Barnett et al. (1954) reported the use of dried and wet whey as a cheap source of carbohydrate to accentuate lactic and acetic acid production. They had previously (1951) used sulfur dioxide in promoting organic acid production. Bolstedt et al. (1941) was quoted by Barnett (1954)

as having used corn meal to increase useful fermentation in grass silages. Working with high protein legume silages Krauss (1941) used hay to increase the carbohydrate and then promoted rapid organic acid production.

Some workers have advocated the use of enzymes, yeasts and bacteria as additives to speed silage fermentation. Kronlik et al. (1955) reported that the microbial population in green plants were absolutely necessary for good silages. Hunter (1917) observed that the population of microorganisms in grass silage increased rapidly within the first two weeks and proposed that in cases where the silage material had been frozen or where workers had suspected a deficiency in microbial content of silage material, deliberate effort should be made to increase them.

(e) Conclusion: From the foregoing partial inventory of related studies, it can be seen that silage fermentation is a well investigated field. It is highly desirable to have a high organic acid production. This phenomenon is a well defined process and the types as well as the levels of the acids produced have much to do with the quality of the silage product. Medium to high quality silages have high lactic and acetic acid levels. Excessive production of butyric acid at the expense of lactic and acetic is a sign of poor quality silage. It has been reviewed how mineral and inorganic acids, fermentable carbohydrates mainly molasses, whey and certain starchy meals have been used to rapidly achieve the desirable fermentation. Some researchers have

used bacterial cultures and others have used yeasts and enzymes to achieve similar purposes in silage making. In the case of bacteria the consensus seems to be that certain rods and cocci are the most prevalent microorganisms in silages. The cocci are believed to disappear as the silage process progresses whereas the population of rods increases. The rods include lactobacilli which are conducive to good fermentation and high lactic acid levels. Many of these researchers have estimated or seem to agree that optimum silage temperature should preferably lie within the 80°F to 100°F range. But this temperature range is subject to variation brought by experimental conditions and the depth and size of the silos from which such temperatures are recorded.

2. Digestibility Trials

When silage has been fermented in the silos, and the material is fed to livestock, the next logical question to ask is how much has fermentation done to hinder or enhance digestibility of the forages? This problem has long been recognized and most silage investigations have included some form of either conventional digestion trial with live animals or used some artificial technique to obtain a criterion on which to assess the digestibility of the particular silage in question. Because most conventional digestion trials with live animals have been expensive, tedious and time consuming, many recent workers, whenever possible, have used artificial techniques to achieve or to attempt to

reach the same goal. Crop scientists, nutritionists and feed manufacturers have used some quick means to have some data or information on the digestibility of their products. In doing so they have tried to approximate natural conditions as much as possible. To achieve this, some have used rumen fluid as inoculum to stimulate microbial growth. Others have used controlled temperatures and some have gone even nearer to nature by using fistulated animals. The proponents of these artificial techniques in forage and feed investigations have advanced reasons why they think these methods are good. They maintain that: (1) the artificial techniques, especially using the nylon or dacron bags, are accurate, rapid and simple for measuring forage qualities. (2) They cut down costs and excessive tine consumption common to conventional trials with live animals. (3) These techniques are suitable means of evaluating forages by small plant breeders whose plots are too small to supply all the forage needed for metabolism trials with large animals like cattle. And (4) that there is a high correlation between conventional and nylon bag technique trials, Van Keuren *et al.* (1962).

Much literature is accumulating on the subject of *in vivo* and *in vitro* digestion of animal feeds. Fina *et al.* (1958) at Kansas Agriculture Experiment Station, Manhattan, carried out artificial techniques in studying rumen digestion *in vivo*. While admitting that it was still difficult to correlate results obtained *in vitro* with those obtained

from actual animals, he maintained that the artificial techniques were necessary and would continue to be. In their experiment, cellulose decomposition was tested by placing tubes containing 500 milligrams of cellulose with and without a rumen inoculum for ten days in the rumen of a fistulated animal. A control tube with distilled water was kept in the rumen through the same period. The procelain tubes used could allow acetic, valeric, propionic, butyric acids, urea and glucose to go through its porous walls. Microbes did not penetrate the tubes and hence the uninoculated tubes remained uncontaminated for twenty-one days. There was no cellulose decomposition in the tube containing cellulose and distilled water after ten days. Thus, under controlled conditions Fina and his group studied cellulose digestion with the help of microorganisms through rumen inoculum. Hughtamen *et al.* (1952) studied artificial cellulose digestions and the results essentially agreed with Finds.

Salsbury *et al.* (1956) studied the rates of cellulose digestion of some plant fractions by rumen microorganisms *in vitro*. The study showed that the liquification of roughages did place a limitation on the digestion of its cellulose. An experiment was carried out in the East African Veterinary Research Center. In this study, Todd *et al.* (1956) investigated the digestibility, chemical composition and nutritive value of certain forage plants at mid-altitude in the tropics. Digestibility varied with the

maturity stage of the grass in question and the season at which the forage was harvested. They also observed that Kikuyu Grass, (Pennisetum Clandestinum) and Rhode grass (Chloris gayana) had high and moderate digestible crude proteins, respectively. Erwin et al. (1959) studied rapid methods of determining digestibility of concentrates and roughages in cattle. They used nylon bags of 4" x 8" tied up to 36 on one chain and inserted in the rumen of a fistulated steer. Four fistulated steers were used for this trial. One of the steers was on a milo, the second on an alfalfa, the third on barley and a fourth on barley straw rations. They observed that the position of the bag in the rumen did not make any difference in the amount of substrate digested. There was a linear decrease in digestibility as weight of material increased in the bags from 10 grams through 24 grams. The greatest decrease of 15 percent in this respect was observed in the milo bags. The variability in digestion within the first nine hours were alfalfa 26-34 percent, straw 16-19 percent, barley 37-54 percent and milo 27-36 percent. In conclusion, the authors mentioned that the technique then used in Arizonian experiment stations was 20 grams in the rumen for nine hours.

In the study of "the dacron bag technique in the evaluation of forages" Hopson et al. (1961) put representative samples of the feed fed to lambs in dacron bags. The grasses used were bromegrass, timothy and the legume alfalfa. The time intervals of the bags in the rumen were 6, 12, 18, 24,

30, 36, and 42 hours. Results showed that only the 36 and 42 hours digestion periods had a high and significant correlation with conventional trials using four years old wethers. Significant digestion rates were found between the three grasses. Alfalfa digestion was detectable between 6 and 12 hours. This was sooner than the rest of the forages. Different digestion rates were found between alfalfa and the other forages when alfalfa was used as the experimental animal feed. On the contrary, there was no significant change in the normal rates of digestion of the four forages when forages other than alfalfa were used in experimental animal ration. Similar results were found by Walker et al. (1956) when they studied the nutritive value of forages for sheep.

Stewart et al. (1961) estimated in vitro volatile fatty acid production from various feeds by bovine rumen microorganisms. They used twelve large mouthed jars and incubated with inoculum at 39-39.5°C in a thermostically controlled water bath. As quoted by Steward, Wasserman et al. (1952) carried out a similar experiment and their findings agreed closely with Stewarts. Schultz et al. (1949) found similar results and in addition reported that propionic acid was glucogenic.

Another study conducted in the area of forage digestibility was at the Washington Agriculture Experiment Station. In this study, Van Keuren et al. (1962) investigated the value of the nylon bag technique for determining

in vivo forage digestibility. The samples of forages used were dried completely at 65°C and were then ground through a 20, 40 and 60 mesh per square inch screens. They used ten grams of feed per bag of 2" x 4½". The nylon string used in fastening the bags to a 3/4" x 8" "lucite" or "Plexiglass" rods had a tensile strength of 75 pounds. These samples were put in pre-weighed nylon bags and dried at 65°C for 24 hours. The bags were labelled with plastic tags. After the bags had been in the rumen of fistulated steers for predetermined time periods they were removed, dried at 65°F for 48 hours in a forced draft oven, removed, crushed and redried for 24 hours. The difference between the dry weight that went into the rumen and that which finally came out of the oven was taken as dry matter digestibility. In some of the trials, twelve bags containing five grams each were tied to one stick. Alfalfa was the substrate and the digestion period was 24 hours. The twelve bags were randomly assigned to positions on the stick. Justifying that it was not necessary to randomize the bags on the sticks, Van Keuren *et al.* quoted Erwin and Elliston (1959) as having found that the position of the bag on the chain did not matter in terms of the amount of substrate digested. However, Niles of the University of Wisconsin at Madison, in a Ph.D. thesis, did find some variations in digestibility according to the levels placed in the rumen.

In discussing their results, Van Keuren et al. stated that there were significant increases in digestibility of orchard grass after 24 hours, alfalfa up to 72 hours and a mixture of both forages after 48 hours. No interaction was found between length of time in the rumen and fineness of grind of the forages in influencing digestibility of the forages.

Although alfalfa still showed slight digestibility at 72 hours, digestion started off earliest, rose until the first 24 hours and then declined. Just as Hopson et al. (1961) found, the diet of the fistulated animal influenced the digestibility of the forages. There was a slight increase in the digestibility of the substrate in bags which were presoaked before immersion into the rumen. The authors explained that some material could have moved in and out of the bag. However they asserted that if this movement were true, then it did so at a uniform rate in all the bags and did not affect accuracy of the results. They concluded that good repeatability was found in the degree and patterns of digestion throughout most of the trials. There were significant increases in digestion of sudan grass and orchard grass after the first 24 hours while alfalfa and ladino clover showed quick initial digestibility before 24 hours and then declined gradually. Fineness of grind did not significantly affect digestibility. Sample sizes affected digestion for comparable periods of time in the rumen. And finally, dietary regime of the animal influenced digestibility.

On the correlation between artificial and conventional digestion trials, Baumgardt *et al.* (1962) evaluated forages in the laboratory. They reported that in comparing conventional and artificial rumen digestion trial methods, the fermentation of carbohydrates by the latter method yielded data which correlated significantly ($P < .05$) with digestibility in the live animal in terms of total digestible nutrients. They also found that forage cellulose digestion in the artificial rumen was closely related to *in vivo* digestibility and results were significantly ($P < .05$) correlated in terms of total digestible nutrients. There were high digestion coefficients for dry matter, organic matter and digestible energy. They also found a very high (0.999) correlation between crude proteins and digestible protein by the artificial digestion technique. In this case of high correlation, the digestible protein could be predicted from the crude protein with a resulting standard error of 0.25 and coefficient of variation of 2.26 percent. Lusk *et al.* (1962) used fistulated steers to suspend small samples of forages in nylon bags in order to determine how coefficients of cellulose digestibility obtained by this method compared with conventional digestion trials. According to them, coastal Bermuda grass had a coefficient of digestion of 63.7 percent with conventional method 61.0 percent with the nylon bag technique at the end of 72 hours. Averages for alfalfa were 56.5 percent digestibility with conventional and 55.2 percent with the nylon bag technique.

Archibald *et al.* (1962) fed a part of the same forage to animals and digested the other part in dacron bags. They got results of digestion from the total feces collection procedure and the dacron bag. Results showed that lower digestibility was obtained with the dacron bags but that the forages which had low digestibility with live animals also had low digestibility with the dacron bag.

A recent work in the area of *in vitro* techniques for comparing cellulose and dry matter digestibility was done by Pettyjohn *et al.* (1964). To the authors this was a simple technique developed for relative comparison of digestibility and dry matter disappearance carried out under a minimum of detrimental conditions. To achieve this, they incubated the forage in the rumen of a fistulated steer which provided normal pH, temperature, and digestible end products.

In their experimental procedure they placed the dry feed in dialysis tubes which were knotted in the middle to provide something like two bags in one. These were then placed in finely perforated plastic cylinders. The cylinders were suspended in the rumen of fistulated Holstein steers for 24 hours. There were four trials in all. After statistically removing all interaction effects the authors found that there was a decrease in coefficient of variation from experiment one to four as a result of improvement in techniques and familiarity with the experimental method. Together with the preliminary results from other cooperative laboratories manned by members of the American Society of

Animal Production, they showed that there was a 30.06 percent cellulose digestion with a standard deviation of 0.41 and total digestibility of 49.45 percent with a corresponding standard deviation of 2.05. They concluded that the results obtained by this experiment compared favorably with contemporary conventional trials. In the fourth trial in which sheep were used for the conventional trial method and where alfalfa and fescue grasses were used, the correlations between *in vitro* and *in vivo* cellulose digestion were 0.584 ($P < .01$) for alfalfa and 0.716 ($P < .001$) for fescue grass. In conclusion, Pettyjohn *et al.* listed some advantages of a study such as this. They were:

(1) It provided actual rumen conditions which other artificial techniques hardly took into consideration or considered necessary.

(2) With the cooperation of other laboratories, several of small forage samples could be evaluated quickly and simultaneously.

(3) The nature of the dialysis tubings used and their encasement in perforated plastic cylinders prevented an excessive bag breakage and allowed a one-way diffusion of dialyzed material; especially the volatile fatty acids and glucose.

(4) The high correlations between the two methods made it possible to predict cellulose, dry matter and energy digestibility of forages if and when fed to normal animals.

They also listed a disadvantage of the method of using live animals. This was that day to day variation in water intake of the animal significantly influenced the amount of forage digested.

Clark (1961) of the Department of Animal Science, Washington State University reported that the nylon bag technique was being studied with the view of perfecting it and devising a formula for calculating total digestibility which would become convenient and acceptable to forage researchers. He however, listed an important assumption underlying the development of such a formula. He stated that for the formula to work, it would be necessary to assume that the fraction of digestion taking place in the rumen (the largest and most active digestion organ in the gastrointestinal tract, Hale et al. (1947) of an animal had a positive correlation to the total digestion. In a paper presented to the American Dairy Science Association, Yang et al. (1962) showed that the correlation coefficient of dry matter disappearance in the nylon bag technique and conventional sheep digestion trial was only +0.14.

From the literature reviewed in the area of digestibility trials some concluding remarks have occurred over and over. Some of these are:

The value of ingested nutrients depends upon what use the body makes of them. Any feed which enters the alimentary canal of the animal and is excreted through the same gut is not utilized. Thus, after ingestion of feed by

animals, the farmer is concerned with the breakdown of such feed into forms which will eventually be absorbed by the proper organs in the animals for the proper nutritional functions. Because of the complicated digestion tract of the ruminant, conduction of digestion trials are tedious and call for 'a total collection' method. Thus, to circumvent this long and involved process and to obtain results, which are relatively reliable, and quick, the nylon bag technique which is being perfected by conscientious workers may assume great importance in this area in the not too distant future.

EXPERIMENTAL PROCEDURE

Experiment 1, Trial 1

A. Silage Fermentation

On June 6, 1964, alfalfa was harvested at the pre-bud stage and chopped into ¼"-½" pieces with a forage chopper and quickly deep frozen at about -20°C. On September 10, 1964, center-cut corn at pre-dent stage was harvested with the same forage chopper, and chopped and frozen in the same manner as the alfalfa. On October 5, 1964, sudan grass (pre-bloom stage) and sudax (fully headed) were harvested with a hand sickle, hauled to the same chopper, chopped and frozen as the alfalfa and corn described above.

On January 24, four miniature galvanized metal silos 6 inches in diameter and 30 inches high, together with 2" x 6" wooden slabs cut to fit the cross section of the hollow silos, were obtained.

The silos were equipped with basketball rubber bladders to render them air-tight and to supply an average pressure of 10 pounds per square inch on the silage material. The pressure was supplied from a tire pump and was channelled through pressure rubber hoses connected with Y-necked glass tubes.

The additives used in this study were: urea containing 42 percent nitrogen, Bio-zyme, a commercial product

obtained from Bio-zyme Products Inc., Cleveland 14, Ohio and Silo Guard from the Stock Food Corporation and distributed by Slim Sherland Co., Bremen, Indiana.[a/]

Each roughage was treated with the various additives in exactly the same way. Usually, the roughage was taken from the freezer the previous night, allowed to thaw overnight in an atmosphere of 75°F. The morning following the thawing, each roughage was treated according to the scheme shown in Table I and as described in the following paragraphs. Also, as can be seen from Table I(a) the sequence of fermentation, arrived at by use of random numbers, was sudan grass first, followed by corn, alfalfa and finally sudax. The application rates of the additives are shown in Table I(b).

Table I. Experimental design

(a) Experimental forages and additives

Additives	Forages			
	Alfalfa	Corn	Sudan	Sudax
P	AP	CP	SP	SXP
Q	AQ	CQ	SQ	SXQ
R	AR	CR	SR	SXR
S	AS	CS	SS	SXS
	3rd	2nd	1st	4th[b/]

a/ As stated in the Introduction, for economy of space these additives will henceforth be referred to according to this coding, Bio-zyme = P, Siloguard = Q, Urea = R, and Control = S.

b/ The numbers appearing in this row are the random sequence of fermentation as arrived at by using a fair dice.

Table I continued .....

Additives	(b) Additive application scheme Code	Application Rate, Percent	Wt./10 kgm
Bio-zyme	P	0.1	10 gm
Silo Guard	Q	0.5	50 gm
Urea	R	0.5	50 gm
Control	S	0.0	0 gm

A black plastic sheet was spread on the floor covering an area of 10'x 10'. The thawed forage was spread over the plastic sheet. All lumps were broken and the forage was thoroughly mixed. After mixing, it was quartered into four representative heaps at each corner of the plastic sheet. The quarters were assigned to treatments at random. Each heap was again homogenized by thoroughly mixing. Exactly 10 kgm samples were weighed from each sample, flattened out into a circular mound of about 30" in diameter and 2" high. The additive (all of which were in powdered forms) was sprinkled uniformly over the entire area of the forage and progressively worked into the entire mass. Two representative samples, one 600 gm and another 200 gm, were taken for dry matter, and pH and chemical determinations, respectively. These samples were put in labelled plastic bags, sealed and quickly deep frozen. The metal miniature silos were then filled with the remaining forage. In filling the silos, great care was taken to make the mass as anaerobic as possible

by tamping down every 6" layer thoroughly with a 2"x 2"x 5" wood piece. When all the material was added, the first wooden block was placed directly above the material. A slightly inflated basketball bladder was placed above this then several more blocks placed over this to occupy the empty space, to about 10" from the silo rim. A basketball bladder was then placed over the last block, followed by the final slab with a half an inch diameter hole bored through the center to admit the rubber hose supplying pressure. Finally, two of the metal rods were put through four holes, two opposite the others to secure the top wooden block. A single filled silo is shown diagramatically in Figure I.

The pressure tubes, connected by Y-necked glass tubes and leading from each silo to a pressure source (tire pump) were set up. One of four one-liter bottles was placed at the base of each silo and two tubes connected to two outlets from the base of each silo led into these bottles which were to receive any run-off. A pressure guage was connected along the pressure path. Finally, pressure of 10 lbs. per sq. in. was applied to and maintained in each silo (see Plates 1 and 2). Since the entire pressure system was uninterrupted from one silo to another, it was feared that a leak or defect in one would affect the others. To safeguard against any such eventuality, metal clamps were placed on the tubes at about 6" from the inlet into the bladder. When pressure was to be applied, the clamps were unscrewed the

proper pressure applied and the clamps were screwed tight again. Plate 2 shows the completed processes and the silos in operation. Pressure readings were taken twice daily, at 8 a.m. and at 6 p.m.

After six days of fermentation, the silage was removed. Samples of 600 gm. and 200 gm. were taken for dry matter and chemical analysis, respectively. These samples were taken in a way that after every three inch layer removed, one hand dip was put into one plastic bag and another into the other. Thus, the silage was uniformly sampled from top to bottom.

B. Chemical Analyses

(a) Dry Matter, pH and Crude Protein

The dry matter content of the silages was determined by drying each sample in duplicate (300 gm. each) in forced draft oven at 100°-105°C for 24 hours. A Beckman pH meter with an external glass electrode was used to determine the hydrogen ion concentration. Each sample was obtained by using 100 gms. of the 200 gm. samples saved for this purpose and for the volatile fatty acids and lactic acid determinations. An extract was prepared for pH determination. A hand scissors was used to cut the silage material as fine as possible. About 25 gm of the chopped forage were then put into a homogenizer and 75 to 100 ml of distilled, deionized water were added. The mixture was homogenized for about five minutes. The extract was obtained by straining the

homogenate through eight layers of cheese cloth into a 50 ml beaker. The volume of the extract was just enough to submerge 3/4 of the electrode. The electrodes were submerged into the extract and the pH was read on the extended scale on the Beckman pH meter for greater accuracy.

Crude protein was determined by using samples of the silages dried for dry matter and ground through a 60 mesh screen in a hammer mill. The method used in this determination was essentially that described by Kjeldahl modified by A.O.A.C. and adapted for use in the Animal Husbandry Laboratory at Michigan State University by B.E. Brent.

(b) Volatile Fatty Acid

Fifteen parts of silage to 45 parts of 0.6N sulfuric acid were mixed in a 100 ml beaker. This mixture was left to stand under refrigeration (37^{o}-39^{o}F) for a minimum of three days. A glass rod was used to churn the mixture in order to homogenize it as much as possible. An extract was made by straining the juice out of the mixture through four layers of cheese cloth. The extract was centrifuged at 16000 r.c.f. for ten minutes. The supernatant was pipetted into 15 ml test tubes and stored under refrigeration until used. A Beckman gas chromatography machine was used in determining the volatile fatty acids. Three microliters were injected per determination and each sample was determined in duplicate (see Plate 3). This was by the method of Wiseman & Irwin (1957.

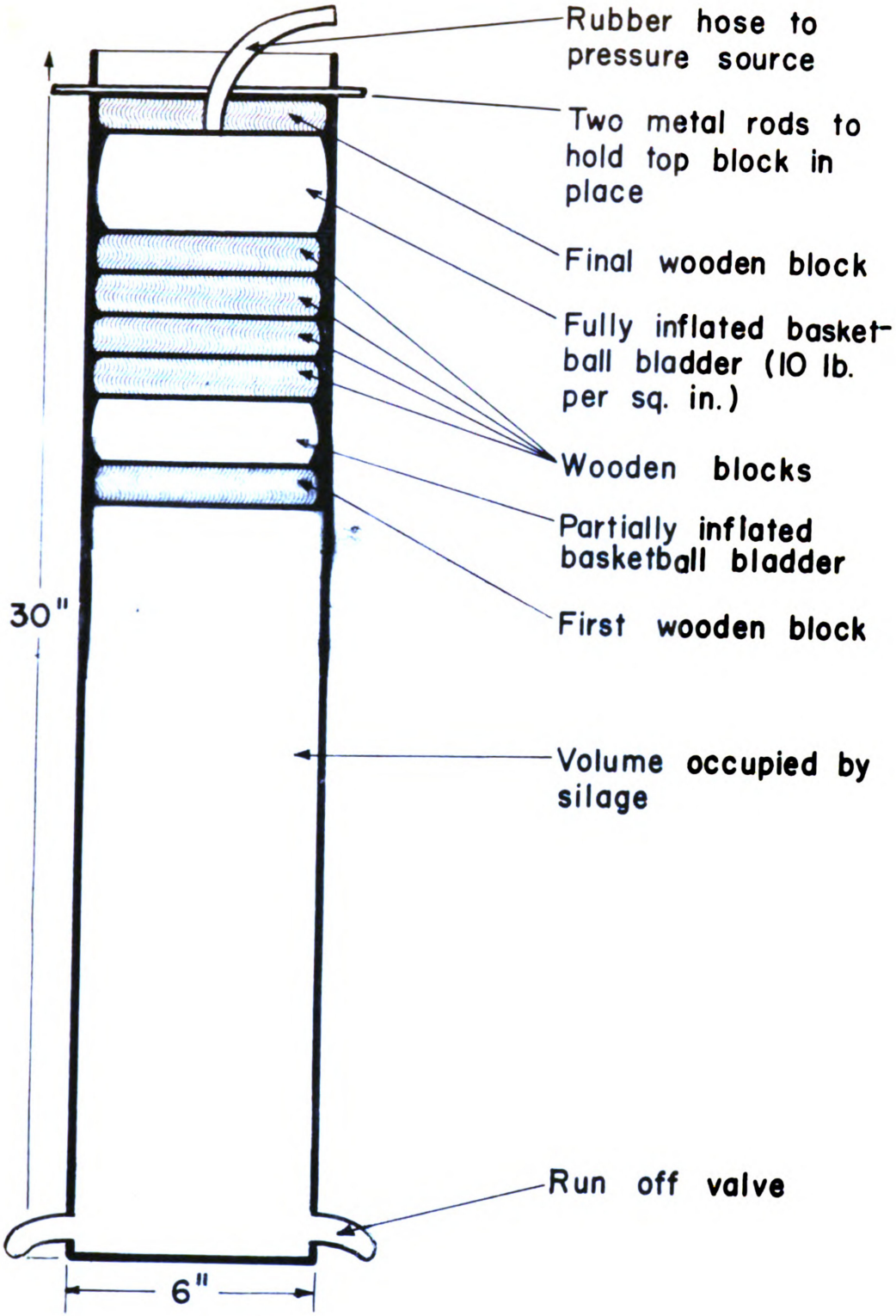


Fig. I: Cross-section of a filled up miniature silo.

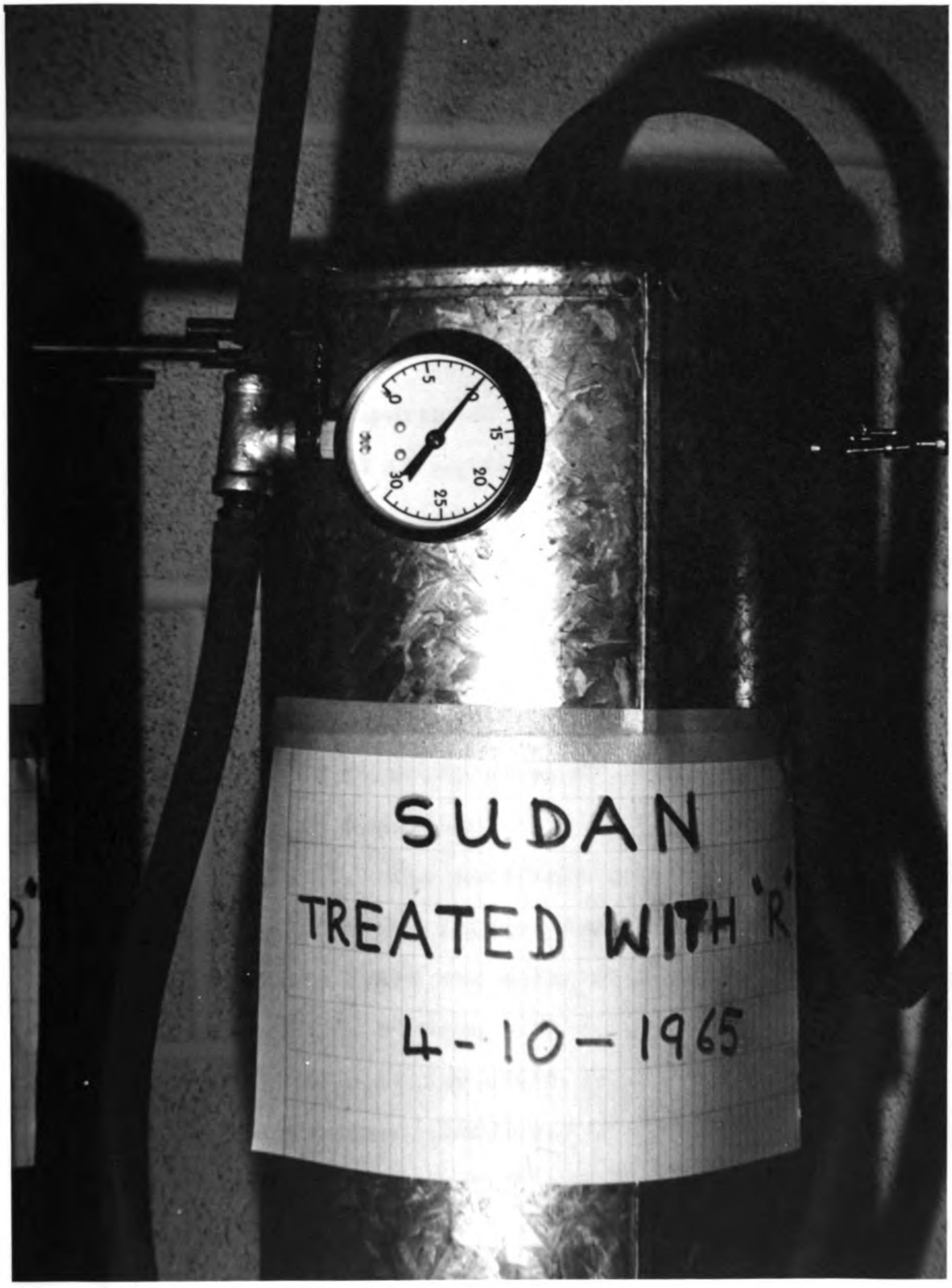


Plate 1: A close-up view of pressure guage in operation showing 10 lb. per square inch.

(c) Lactic Acid

Lactic acid was determined according to the procedure outlined by Barker and Summerson (1941). The only deviation from the outline was that trichloroacetic acid was not used to precipitate the protein because several trials had shown the lack of precipitable protein.

EXPERIMENT I, TRIAL II

Trial II of Experiment 1, was an exact replicate of all the steps outlined in Trial I. It is therefore to be understood that what applied to any of the steps outlined in the procedure to Trial I will necessarily follow in Trial II.

EXPERIMENT II: DIGESTIBILITY

The second experiment involved use of a modified nylon bag technique to study rumen dry matter digestion of the silages made in Experiment I. The experimental design is shown in Table II. The materials used in this trial were:

1. Feed samples from the silages made from alfalfa, center cut corn, sudan grass and sudax treated with additives.
2. Nylon bags of 2"x 5" sewn with a "French seam"- sewn so that the cut edge of the cloth is not exposed even if the bags were turned inside out - using nylon thread and nylon cloth with 105 strands per linear inch.
3. Dextrose dialysis tubings of about an inch diameter and 6 inches long.
4. Nylon fishing line with a tensile strength of 45 pounds.

Plate 2: Complete set up for a typical six day run

Plate 3: Assistant injecting 3 millimoles of sample into a gas chromatograph for volatile fatty acid determination.

Table II. Experimental design - digestibility trial

Silage	Additive	Code	Application Rate %	Hours in rumen 12	24	36
				Number of bags		
Alfalfa	Bio-zyme	P	0.1	2	2	2
	Silo Guard	Q	0.5	2	2	2
	Urea	R	0.5	2	2	2
	Control	S	0.0	2	2	2
Corn	Bio-zyme	P	0.1	2	2	2
	Silo Guard	Q	0.5	2	2	2
	Urea	R	0.5	2	2	2
	Control	S	0.0	2	2	2
Sudan	Bio-zyme	P	0.1	2	2	2
	Silo Guard	Q	0.5	2	2	2
	Urea	R	0.5	2	2	2
	Control	S	0.0	2	2	2
Sudax	Bio-zyme	P	0.1	2	2	2
	Silo Guard	Q	0.5	2	2	2
	Urea	R	0.5	2	2	2
	Control	S	0.0	2	2	2

5. Three metal rings of about 3" in diameter made from heavy wire and encased in transparent plastic tubes to prevent rusting when in contact with rumen fluid or liquid.
6. Glass beads, used to equalize weight of the control bags with those containing the experimental silages.
7. A fistulated Holstein steer of about 18 months old.

Procedure

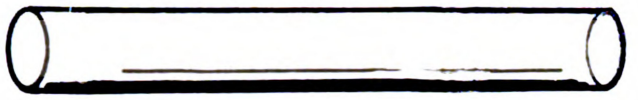
About 15 gm. samples from the same dried silages used in Kjeldahl nitrogen determination in Experiment I were oven dried at 101°C for five hours. Pieces of dialysis tubings, 6" long were wetted in a beaker of warm water and a knot made at one end (see Fig. II) to make an open sac. The nylon bags were numbered in two positions using an ordinary black ball point ("BIC") pen. A knotted dialysis tubing was placed in each nylon bag and oven dried at 101°C for 12 hours. When they attained constant weights, the bags were removed from the oven and quickly put into a large desiccator. Vacuum suction was applied to create a vacuum in the desiccator. The desiccator and contents were allowed to stand for thirty minutes. Each bag was weighed and the weight recorded against the identification number of the bag.

After weighing the bags each dialysis tubing was wetted, blown into a cylindrical sac and approximately two grams of silage samples, dried to constant weight and stored in a desiccator under vacuum, were put in the tubing and the other end knotted to obtain a sealed bag. Each tubing

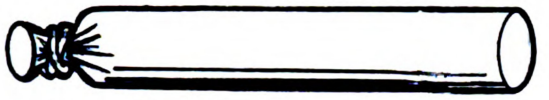
containing the silage was placed into the same nylon bag with which it had previously been weighed. Care was taken so that there was no mixing of labelled bags and tubing. The gross (nylon bag and dialysis tubing and feed sample) as well as net (feed sample alone) weights of each bag were recorded. There were six such bags for each sample of silage. There were also six bags of glass beads treated exactly like those of silage samples. Thus, in one run of digestion, six bags each of four silages plus six bags of control (glass beads) were used.

Two bags of each silage plus two of the control, were tied to a metal ring leaving about three inches of string between the ring and the bags (see Plate 4). There were a total of eighteen bags on each ring made up of eight different silages, and two controls. About 18" of a triple woven nylon string (the same used in tying the bags) was attached to each ring carrying the 18 bags. Three such rings each carrying 18 bags and all making a total of 54 were attached to a single thread of short piece of the nylon string. A thick knot was made at the end of the short string not attached to the three triple woven string carrying the rings.

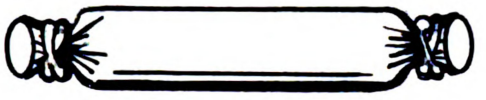
The fistulated Holstein steer which had been on a constant ration of alfalfa hay fed ad libitum, trace mineral salt and water for 7 days prior to the experiment and continued on the same until the experiment was over, was used. At 8 p.m., three hours after the steer was fed, the three rings

1. 

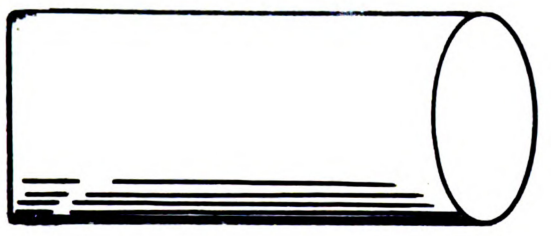
A 6" piece of dialysis tubing.

2. 

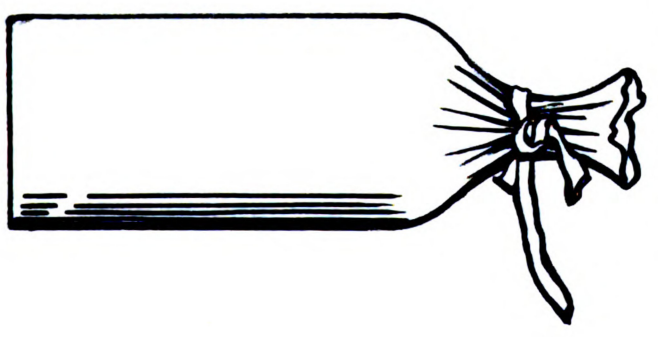
Same tubing with one knotted end.

3. 

Same tubing with both ends knotted containing silage sample.

4. 

A 2"x 5" nylon bag.

5. 

Same nylon bag containing #3 and mouth tied with string ready for ring attatchment.

Fig. II: Progression in preparing the nylon bag.

carrying eighteen bags each were carefully placed into the rumen through the fistula. The three rings were distributed so as to avoid entanglement during rumen churning. The small string was held at the window and the lid was carefully screwed in place so that the big knob was outside to prevent the string holding the bags from slipping completely into the rumen.

At 8 a.m. the next day, 12 hours after placing in the rumen, one ring was removed and flushed with warm tap water from a hose as shown in plates 5 and 6 for ten minutes. The bags were then quickly deep frozen. The second ring of bags was removed from the rumen 24 hours and the third 36 hours after they were introduced into the rumen. The same flushing and freezing procedure was followed as with the first ring. After the third ring of bags was out, they were taken to a forced draft oven at 101°C and dried for 18 hours. The rest of the silages in the first and second runs went through exactly the same procedure.

After they were dried for 18 hours, and cooled in a desiccator under vacuum for thirty minutes, each bag was weighed and the weights recorded. Percentage digestibility was calculated according to the following formula:

$$\% D = G_i - \frac{(G_o + C_f)}{N_i} \times 100$$ where

D = Digestibility, Gi = Gross input (Tare + Undigested feed)
G_o= Gross output (Tare + partially digested feed);
C_f= Correction factor ($\pm$ change of weight in beaded bags)
N_i= Net input (actual sample weight).

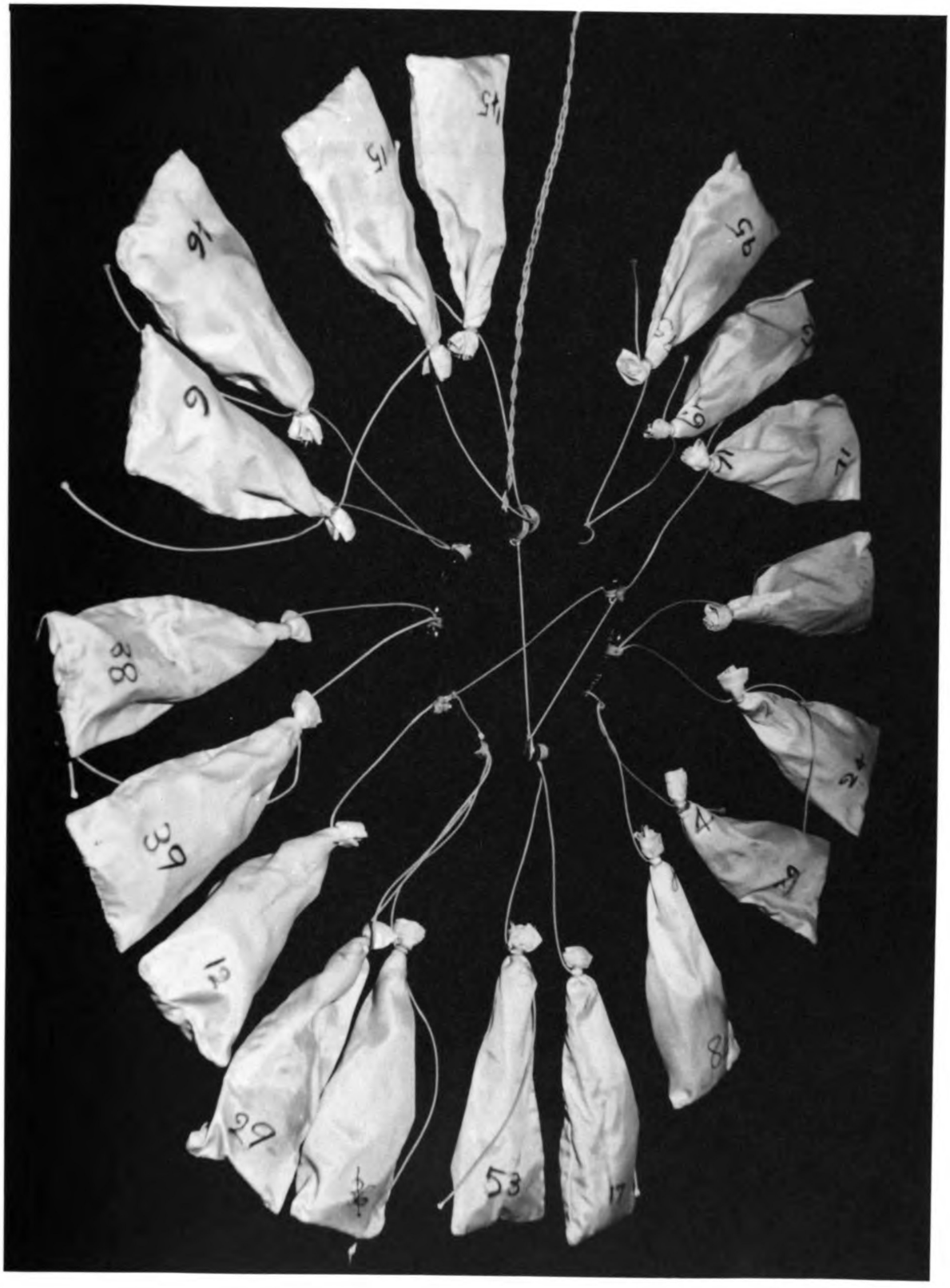


Plate 4: Nylon bags containing a set of 18 forage samples; 16 experimental and 2 controls; arranged on a metal ring previous to placement into the rumen.

The final digestibility figure for any of the treatments in any given period was therefore an average of the two bags of that particular treatment.

The results were analyzed using analysis of variance methods according to Snedecor (1956) and Scheffé (1959).

Plate 5: Flushing one ring of bags with tap water just after removal from the rumen.

Plate 6: A group of bags on one ring, left to drain after flushing.

RESULTS

Overall Effect of Fermentation

The results showing the overall effect of fermentation are given in Table III. Here, every other thing (additives and variations due to kind of forages) is assumed to be held constant and only fermentation per se is considered. Except for lactic acid, each of the variables was determined prior to and again after fermentation. The standard deviations are included in this table to show how much each of the individual values deviated from the mean in each case. Apart from the pH with a standard deviation of .35 from the mean of 5 before and .23 from a mean of 3 after fermentation, it can be seen that every other result had a very high standard deviation. This is, of course, expected since some conditions which affected some of these values were originally of different magnitudes in the different forages. An example here is the protein content of alfalfa, which usually averages about twice as high as that of corn.

The data in Table III show a slight increase in dry matter from 29.23 before to 31.23 after fermentation. This was apparently due to the pressure of 10 pounds per square inch exerted on the silage during fermentation which expressed some of the juice.

There was a slight decrease (.29%) in crude protein due to fermentation, and a distinct drop in pH.

Table III. Overall effect of fermentation on nutrient content, pH and digestibility of all silages[a/]

	Dry Matter %	Crude Protein %	pH	Acetic acid[b/]	Propionic acid[b/]	Butyric acid[b/]	Lactic acid[b/]	Digestibility %
Mean before fermentation	29.23	11.89	5.33	9.69	10.44	1.10	-	13.89
Standard Deviation	7.94	4.34	.35	3.89	3.15	1.83	-	8.46
Mean after fermentation	31.23	11.60	3.70	72.23	5.47	1.36	193.58	7.47
Standard Deviation	8.21	4.21	.23	29.08	6.03	1.94	30.37	4.46

a/ Here no account is taken of the variations in the various different forages or the effects of the various additives, as regards these variables. The only variable whose effect is being measured is fermentation.

b/ All acids measured in micromoles per gram of silage on dry matter basis.

There were very significant increases in the volatile fatty acids as a result of fermentation. Acetic acid significantly increased as a result of fermentation from 9.69 to 72.23 micromoles per gram of silage. Propionic acid did not follow this trend. Instead, it decreased from 10.44 to 5.47 micromoles per gram. The apparent decrease in propionic acid after fermentation could not be explained. However examination of the data for individual forages (not shown in Table III) revealed considerable variation in propionic acid production before and after fermentations.

After massing everything in one table as in Table III, it was seen that some pecularities belonging to each variable were obscured. Hence, tables were made for each of the variables so as to show some of these distinctive features.

In this case, the effect of the additives played an important part. Since most of the study had to do with additive effects on the various silages through the process of fermentation, all but the data in Tables I through III are concerned with the results as determined after fermentation.

Additive Effect on Dry Matter

The results in Table IV show the additive effect on dry matter conservation through fermentation. Since, due to original differences in moisture content, one would expect a difference in dry matter value among the different forages, no attempt was made here to interpret these values shown as average at the extreme right of the table. However, the values under the column of each additive are considered. Analysis of variance showed no significant differences in additive effect on the dry matter of the forages. It can be seen in Trial I that there were some slight differences among additive columns. In this trial, all additives resulted in slight increases in dry matter values over the control. Here addition of Silo Guard resulted in slightly higher dry matter than the rest, with an average of 36.35. The control(s) showed the least value (34.46) of dry matter. This trend was not shown in Trial II, which was a replicate of Trial I. The control values were slightly higher than those of some of the additives. There was virtually no difference in additive effect. However there was a clear discrepancy between the results of Trials I and II. More dry matter seemed to have been conserved in Trial I than in II.

Table IV. Additive effect on dry matter during fermentation

Forages	Trial I Additives				
	P	Q	R	S	Ave.
Alfalfa	41.78	41.83	40.08	39.00	40.7
Corn	38.60	38.95	36.50	35.91	37.5
Sudan	21.57	21.24	20.25	21.93	21.2
Sudax	41.95	43.38	43.93	41.00	42.6
Ave.	35.97	36.35	35.19	34.46	

Forages	Trial II Additives				
	P	Q	R	S	Ave.
Alfalfa	27.35	27.79	28.34	28.20	27.9
Corn	32.74	32.49	32.57	32.32	32.5
Sudan	20.00	19.88	19.90	18.40	19.60
Sudax	26.62	28.04	27.54	29.40	27.9
Ave.	26.67	26.93	27.08	27.08	

The overall average dry matter of Trial I was 35.49, compared to 26.94 of Trial II. This difference in dry matter could not be explained in terms of any variation in procedure.

Additive Effect on pH

The pH depressing effect of the additives is shown by the data in Table V. It may be said at this point that lower pH values are desirable for good quality silage and all values shown in Table V are within this range. Analysis of variance showed values from silages treated with Urea (R) to be significantly ($P < .05$) higher than those obtained from the control (S) and other additives; Silo Guard (Q) and Bio-zyme (P). Both Silo Guard and Bio-zyme consistently resulted in values lower than the control in both trials.

There were also significant ($P < .05$) differences between the pH values from the different forages. The results showed that the fermentation of alfalfa in both trials resulted in higher pH values (4.03, 3.91) than the other forages. Sudax produced the second largest pH values (3.71, 3.78) followed by corn (3.66, 3.54) and finally by sudan (3.61, 3.40) in both trials.

Additive Effect on Crude Protein

The results showing additive effect on crude protein are presented in Table VI. Here, as already mentioned in the overall effect of fermentation, it is clear that there are

Table V. The pH depressing effect of additives as measured after fermentation

Trial I

Forages	Additives				
	P	Q	R	S	Ave.
Alfalfa	4.00	3.91	4.25	3.95	4.03[b]
Corn	3.65	3.58	3.79	3.63	3.66[d]
Sudan	3.10	3.27	3.55	3.75	3.42[d]
Sudax	3.79	3.63	3.70	3.74	3.71[d]
Ave.	3.64[d]	3.60[d]	3.82[c]	3.77[d]	

Trial II

Forages	Additives				
	P	Q	R	S	Ave.
Alfalfa	3.92	3.64	4.12	3.95	3.91[b]
Corn	3.58	3.34	3.61	3.63	3.54[e]
Sudan	3.10	3.27	3.61	3.61	3.40[e]
Sudax	3.75	3.70	3.96	3.70	3.78[d]
Ave.	3.59[e]	3.49[e]	3.83[c]	3.72[d]	

b,c,d,e, Means bearing different superscript letters on the same line and column significantly differ ($P < .05$).

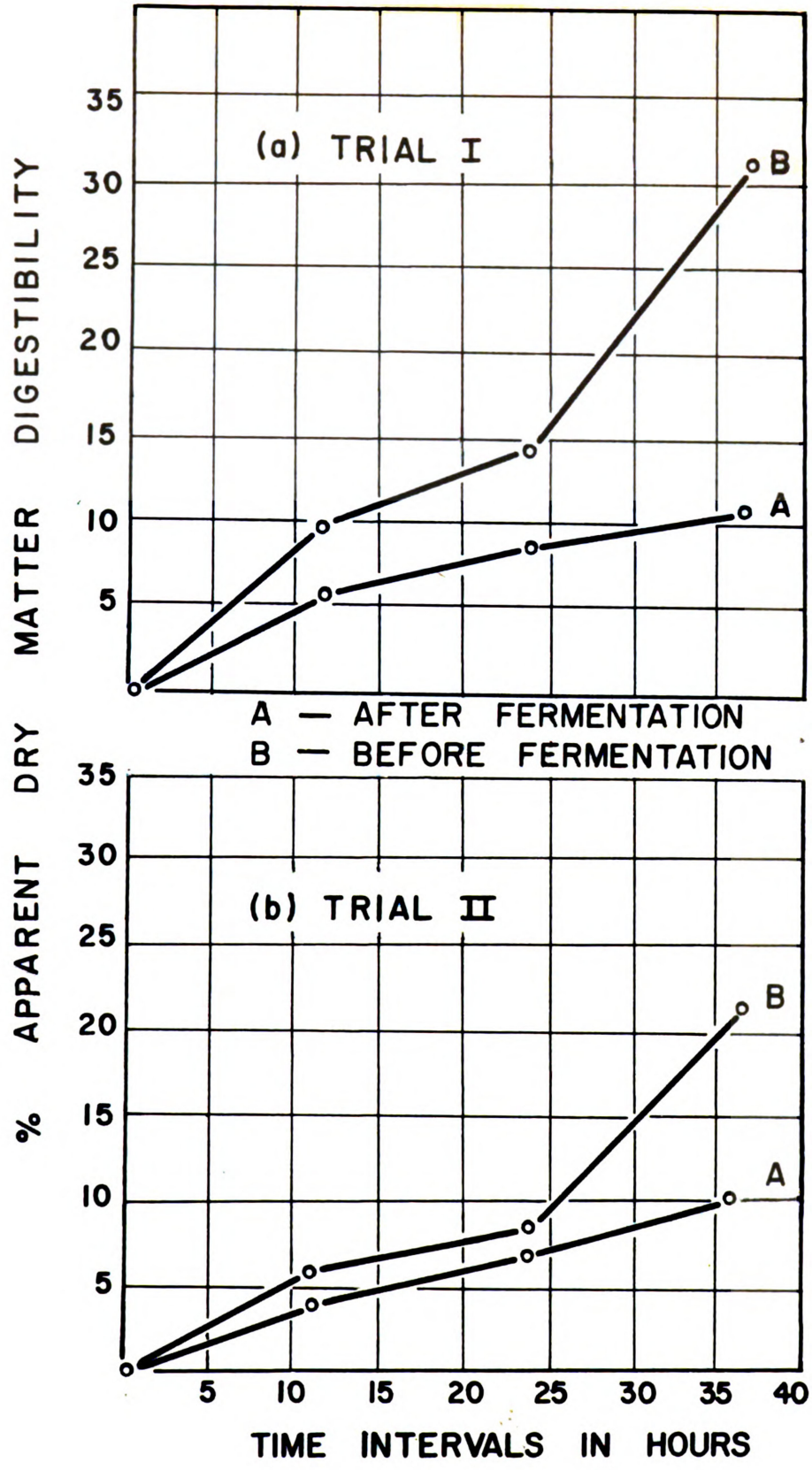


Fig. III: The effect of time on apparent dry matter digestion.

inherent differences in protein content of the forages. Thus, no attempt was made to subject forage effect on protein retention through fermentation to statistical analysis. However, analysis of variance show ed the differences in additive effect on this nutrient to be significant. In both trials, protein values from additive (R) were significantly higher ($P < .05$) than the control and other additives, but these were expected because the additive R was urea (42%N) applied at the rate of .5%. On the other hand, adding Biozyme (P) resulted in protein values almost equal to the control (S) while Silo Guard (Q) had no consistent effect; lowering protein values in Trial I and slightly increasing it in Trial II.

Additive effect on volatile fatty acids

(a) Acetic. The results showing the production of acetic acid are presented in Table VII. Analysis of variance showed some significant additive effect on this acid. Trial I showed that urea (R) significantly ($P < .05$) increased acetic acid production over the control including other additives. Differences in acetic acid production due to forages were statistically significant ($p < .05$). When confident interval limits using the Scheffé (1959) method were carried out between the mean acetic acids due to each forages, it was shown that the mean alfalfa was significantly ($P < .001$) different from the rest; and that of sudax was also significantly ($P < .05$) greater than those of sudan and corn.

Table VI. Additive effect on crude protein after fermentation[a/]

Forages	Trial I Additives				
	P	Q	R	S	Ave.
Alfalfa	17.32	16.66	19.18	17.66	17.71
Corn	9.89	9.34	11.38	10.35	10.24
Sudan	10.90	9.90	11.20	10.15	10.54
Sudax	7.06	7.25	9.62	6.96	7.72
Ave.	11.29[c]	10.79[c]	12.85[b]	11.28[c]	

Forages	Trial II Additives				
	P	Q	R	S	Ave.
Alfalfa	17.90	18.22	20.15	18.48	18.69
Corn	7.63	8.34	10.24	7.51	8.43
Sudan	10.16	10.23	14.36	10.27	11.26
Sudax	7.21	7.90	10.54	7.19	8.21
Ave.	10.73[c]	11.17[c]	13.82[b]	10.86[c]	

a/ Results given as percent of total dry matter.

b,c. Means bearing different superscript letters on the same line differ significantly ($P < .05$).

Table VII. Additive effect on acetic acid production during fermentation in micromoles/gram[a/]

Trial I

Forages	Additives P	Q	R	S	Ave.
Alfalfa	131.4	137.7	150.9	134.0	138.5[e]
Corn	58.3	57.0	61.8	51.6	57.2[c]
Sudan	51.2	44.7	43.8	44.6	46.1[c]
Sudax	76.6	80.9	76.6	69.1	75.8[b]
Ave.	79.37[c]	80.1[c]	83.3[b]	74.8[d]	

Trial II

Forages	Additives P	Q	R	S	Ave.
Alfalfa	52.6	47.8	48.0	59.7	52.0[d]
Corn	66.8	52.5	78.3	71.0	67.2[c]
Sudan	70.1	70.1	97.3	98.7	84.1[b]
Sudax	57.4	52.2	66.4	52.2	57.1[d]
Ave.	61.7[c]	55.7[c]	72.5[b]	70.4[b]	

a/ On dry matter basis

b,c,d. Means on the same line and column bearing different superscript letters differ significantly ($P < .05$).

e, Significantly different ($P < .001$).

Considering the forage effect on acetic acid production, it can be seen that the values for alfalfa in Trial I almost doubled those in Trial II. There was no way the experimenter could account for such obvious differences.

There was a consistent drop in acetic acid production from Trial I to II in terms of additive effect. Urea (R) which showed the highest value of 88.3 micromoles per gram of dry matter showed the highest value (72.5) in Trial II. The positions reversed for Silo Guard. Its addition to silage resulted in the second largest value (80.1) in Trial I while in II it resulted in the lowest acetic acid value (55.7 micromoles per gm.). Both reversion in acetic acid production by certain additives and forages from one trial to the other could not be explained.

(b) Propionic Acid. Results for propionic acid are presented in Table VIII. Generally, the production of propionic acid was far less than that of acetic and lactic. There was no consistent pattern in the production of this acid either among the forages or additives. Alfalfa showed no production in Trial I while in II it showed about 4 micromoles per additive. Corn averaged 6.9 in Trial I, and up to 11.4 in II. Sudan which produced most of this acid in Trial I showed no production in P, R, and S treatments in Trial II. The variations being so obvious as they were, and the total production of the acid being as low as it was in this case,

Table VIII. Additive effect on propionic acid production during fermentation in micromoles/gram[a/]

Trial I[b/]

Forages	Additives				
	P	Q	R	S	Ave.
Alfalfa	0.0	0.0	0.0	0.0	0.0
Corn	4.7	2.92	7.18	12.69	6.9
Sudan	12.56	14.92	16.88	18.40	15.7
Sudax	10.58	0.0	0.0	0.0	2.64
Ave.	7.0	4.5	6.0	7.8	

Trial II[b/]

Forages	Additives				
	P	Q	R	S	Ave.
Alfalfa	7.76	4.14	3.18	3.66	4.7
Corn	11.53	11.35	9.23	13.45	11.4
Sudan	0.0	9.85	0.0	0.0	2.71
Sudax	0.0	0.0	0.0	0.0	0.0
Ave.	4.7	6.3	3.1	4.3	

a/ On dry matter basis

b/ Differences too obvious to warrant statistical analysis.

no attempt was made to analyze the results statistically. It might be mentioned at this point that butyric acid was also determined. The production of this acid was far below that of propionic and was so irregular among additives and forages that it defied any intelligent presentation in a table.

(c) Lactic Acid. Lactic acid was the major acid produced from the fermentation. Its production doubled that of acetic, the second major acid. Table IX shows the values of this acid. Considering the effect of additives, analysis of variance showed significant ($P < .05$) differences between the treatments and the controls in Trials I and II. Apparently, urea (R) was the only additive which significantly influenced lactic acid production. It can be seen in the table, except for one case in Trial II, that this increase was consistent over the control (S) and higher than the other two additives in all cases. On the other hand, Silo Guard (Q) and Biozyme (P) produced lactic acid in the neighborhood of the control, slightly higher in some cases and slightly lower in others, but forming no consistent pattern. The average lactic acid was slightly higher in the second trial than in the first when additive effect was considered.

Considering lactic acid production in terms of the various forages, the results showed that there was statistically significant differences in both trials. In Trial I, it was significant at the five percent level of probability. The unusually high probability ($P < .01$) was brought about in Trial II by very low values for alfalfa. Unlike the acetic

acid, average values for lactic acid were consistently higher for Trial II than they were for I. On the forage side, it may be seen that alfalfa which was lowest in Trial I was equally the lowest in Trial II. Sudan had the second highest value (197.6) in Trial I and in II, it had the highest, 223.0 micromoles per gram. The average values for all additives and forages were higher in Trial II than in I, except for alfalfa.

Digestibility Trial. The second experiment dealt with forage digestibility. Detailed results are presented in Tables X (a) and (b) and XI (a) and (b). In both tables, digestion results included those carried on fermented as well as unfermented forages. In this experiment, the forage additive and interaction effects between both were statistically significant ($P < .05$) on both fermented and unfermented silages. In the first 12 hours, all the three factors (forages, additives, and interactions showed significant ($P < .01$) differences in the way each affected digestion. The same was true for the 24 hour period in the unfermented forages. During the 36 hour period, the forage effect showed significant ($P < .1$) differences in digestibility.

The effect of time interval was outstanding. The results showing this are the averages in the columns on the extreme right of Tables X and XI. In both tables, and in each case which shows pre- and post-fermentation figures, it can be seen that percent digestibility increased with time. In the case of pre-fermentation in both tables, the amount

Table IX. Additive effect on lactic acid production during fermentation in micromoles/gram[a/]

Trial I

Forages	Additives				
	P	Q	R	S	Ave.
Alfalfa	160.0	144.9	174.4	155.7	158.8[e]
Corn	172.6	170.1	209.2	194.0	186.5[c]
Sudan	183.2	192.5	201.1	213.7	197.6[cb]
Sudax	230.6	190.3	197.8	182.7	200.4[b]
Ave.	186.6[c]	174.5[d]	195.6[b]	186.5[c]	

b,c,d. Means on the same line bearing different superscript letters differ significantly ($P < .05$).

Trial II

Forages	Additives				
	P	Q	R	S	Ave.
Alfalfa	155.5	155.5	204.1	114.7	157.5[e]
Corn	214.2	218.0	194.0	246.2	218.1[bc]
Sudan	229.3	216.7	241.9	204.1	223.0[b]
Sudax	210.4	191.3	241.7	184.0	206.9[c]
Ave.	202.4[c]	195.4[c]	220.4[b]	187.3[d]	

a/ On dry matter basis

b,c,d, Means bearing different superscript letters on the line and column differ significantly ($P < .05$).

e, Significantly less ($P < .01$).

digested during the first 12 hours was approximately half that digested during the 24 hours which in turn was about half that digested during the 36 hour periods. The results on the fermented material did not follow this pattern of doubling the previous figure, but nevertheless, they showed the same trend of the direct relation between increase in time and corresponding increase in percent dry matter digestion. These results are also shown graphically in Figure III.

There was an unusual observation on the digestion trial. The results, as seen in detail, in Tables X and XI and again as presented in Table XII, showed that the unfermented material was more digestible than the fermented. There seemed to be no precise explanation to this. However, the author attributed this apparent discrepancy to one factor. This could have been due to the drying of the ground silages. Both fermented and unfermented forages went through the same drying and preparation processes. However, because the fermented material had undergone considerable breakdown resulting in large amounts of volatile and non-volatile fatty acids, primarily acetic and lactic, these nutrients apparently escaped during the drying. Thus, the residual dry matter in the fermented forages could have been less digestible.

Another discrepancy showed up in the results between Trials I and II. Generally, there appeared to be more dry matter digested in Trial I, both in fermented and unfermented silages, than in Trial II. Tables X and XI reveal an average

Table X a Dry matter digestibility as affected by time intervals and additive treatment

Time	TRIAL I: Before fermentation a/																Ave. all forages
	ALFALFA				CORN				SUDAN				SUDAX				
	P	Q	R	S	P	Q	R	S	P	Q	R	S	P	Q	R	S	
12	13.4	16.9	14.9	12.6	8.0	8.6	8.7	9.4	10.8	12.0	10.1	12.2	2.9	3.2	3.2	2.2	9.3
24	16.2	25.3	21.7	21.8	18.1	15.7	14.2	26.4	12.3	14.8	12.2	13.4	3.9	5.8	7.6	3.3	14.5
36	23.5	53.9	52.9	50.1	43.7	47.7	45.6	36.7	19.2	33.4	29.3	20.5	8.7	10.6	11.7	9.0	31.0
Ave.	24.1^{e}	32.0^{bc}	29.8^{c}	28.1^{c}	23.2^{c}	24.0^{b}	22.8^{c}	24.1^{b}	14.1^{d}	20.0^{b}	17.2^{c}	15.3^{d}	5.1^{c}	6.5^{b}	7.5^{b}	4.8^{c}	

a/ Measured as a percentage of total dry matter of each sample put into the rumen.

b, c, d Means under each forage column bearing same superscript letters do not differ significantly ($P<.05$).

e Significantly less ($P<.01$).

Table X b Dry matter digestibility as affected by time intervals and additive treatment

	TRIAL I: After fermentation [a]																
	ALFALFA				CORN				SUDAN				SUDAX				Ave. all forages
Time	P	Q	R	S	P	Q	R	S	P	Q	R	S	P	Q	R	S	
12	8.9	12.7	12.1	12.3	2.9	2.6	2.8	3.0	4.0	6.2	5.4	4.2	3.4	2.5	3.7	2.7	5.6
24	10.7	15.5	16.7	15.1	5.1	5.8	5.5	5.2	7.3	7.7	14.4	4.8	3.8	5.6	5.6	4.6	8.3
36	16.1	29.2	22.3	*	9.2	6.4	4.3	5.6	7.7	9.4	10.1	11.8	4.4	7.6	7.0	8.5	10.6
Ave.	11.9[c]	19.1[b]	17.0[b]	13.7[c]	5.7[b]	4.9[c]	4.2[b]	4.6[b]	6.4[c]	7.7[c]	9.9[b]	7.0[c]	3.9[c]	5.2[b]	5.4[b]	5.3[b]	

* Bag punctured and averages calculated minus this value.

a/ Measured as a percentage of total dry matter of each sample put into the rumen.

b, c Means under each forage column bearing same superscript letters do not differ significantly ($P<.05$).

of 9.3 vs. 5.7 for the 12 hour period, 14.5 vs. 8.3 for the 24 hour, and 31 vs. 21.6 for the 36 hour periods for the unfermented silages in Trial I and II, respectively. The same trend, although not to the same magnitude, showed up in the fermented silages.

There was no consistent additive effect on dry matter digestibility. The data in Table XII and again as presented in Figure IV show that urea (R) and Silo Guard (Q) significantly ($P < .05$) increased percent dry matter digestion of alfalfa and corn over the control (S), on unfermented forages. Both additives also showed increases in dry matter digestion on unfermented sudan and sudax but these were not significant. Except in sudax where Bio-zyme (P) increased forage digestibility over the control (S), it consistently lowered digestibility of the other forages below the control.

A point of crucial consideration was the effect of the additives on the fermented silages. Silo Guard (Q) and urea (R) significantly ($P < .05$) increased dry matter digestion of alfalfa. Bio-zyme (P) slightly depressed alfalfa dry matter digestion below the control (S). There was virtually no additive effect on the digestion of corn silage. In fact, all the additives lowered corn silage digestion by 1% below the control. Only urea (R) slightly increased percent dry matter digestibility of sudan and sudax over the control. Bio-zyme (P) and Silo Guard (Q) actually decreased dry matter digestibility of both silages below the control.

Considering additive effect on percent dry matter digestion of both fermented and unfermented silages, the

Table XI a Dry matter digestibility as affected by time intervals and additive treatment

	TRIAL II: Before fermentation a/																
	ALFALFA				CORN				SUDAN				SUDAX				Ave. all forages
Time	P	Q	R	S	P	Q	R	S	P	Q	R	S	P	Q	R	S	
12	3.2	3.2	5.0	1.8	7.3	8.1	9.2	9.7	7.9	6.4	6.2	6.3	2.8	4.0	7.3	3.0	5.7
24	6.6	6.9	5.4	4.6	13.5	12.0	11.7	13.4	8.6	6.1	7.2	8.6	11.9	5.9	5.4	4.3	8.3
36	11.9	13.9	20.9	11.5	21.8	26.3	31.0	17.3	11.1	11.8	11.9	11.3	5.5	9.6	9.3	7.5	21.6
Ave.	7.2^{cd}	8.0^{bc}	10.4^{b}	5.6^{d}	14.2^{dc}	15.5^{c}	17.3^{b}	13.5^{d}	9.2^{f}	8.1^{b}	8.4^{b}	8.7^{b}	6.7^{b}	6.5^{b}	7.3^{b}	5.0^{c}	

a/ Measured as a percentage of total dry matter of each sample put into the rumen.

b, c, d Means under each forage column bearing same superscript letters do not differ significantly ($P<.05$).

Table XI b Dry matter digestibility as affected by time intervals and additive treatment

	TRIAL II: After fermentation [a/]																
	ALFALFA				CORN				SUDAN				SUDAX				Ave. all
Time	P	Q	R	S	P	Q	R	S	P	Q	R	S	P	Q	R	S	forages
12	7.6	7.8	7.4	7.0	4.9	1.9	1.5	3.3	3.3	3.0	4.1	3.3	2.4	2.9	3.2	2.7	4.2
24	11.0	11.2	12.0	11.1	5.1	4.6	2.0	7.0	4.7	5.0	9.7	8.8	3.2	4.3	4.7	3.4	6.7
36	19.5	17.8	13.3	18.3	*	8.3	12.3	11.7	12.5	9.8	6.6	9.3	8.4	*	6.8	5.6	10.0
Ave.	12.7^{b}	12.3^{b}	10.9^{c}	12.1^{b}	5.0^{b}	4.9^{b}	5.3^{b}	7.3^{c}	6.9^{b}	5.9^{b}	6.8^{b}	7.1^{c}	4.7^{b}	3.4^{c}	4.8^{b}	3.9^{b}	

* Bag punctured and averages calculated minus this value.

a/ Measured as a percentage of total dry matter of each sample put into the rumen.

b Means under each forage column bearing same superscript do not differ significantly ($P<.05$).

results showed that Silo Guard (Q) and urea (R) consistently increased percent dry matter digestion of all unfermented forages, and did the same on fermented alfalfa. All additives were inferior to the control in fermented corn and only urea (R) slightly increased the percent dry matter digestion of fermented sudan and sudax.

It could have been interesting to see which forage was most digestible. But considering that they were of different stages of maturity and some were of higher cellulose content than others on account of species and maturity, the researcher thought this would not serve any useful purpose; hence, this aspect was not considered.

As it was described in the experimental procedure, attempts were made to maintain a constant pressure in the silos during the process of fermentation. No attempt was made to correlate these pressure values with other variables in the silage fermentation. The figures are presented in Table XIII. One important fact is shown in the average results of Trials I and II. Except for the silo with alfalfa treated with 'S' in Trial II, all pressures showed at least 9.5 pounds per square inch. In Trial I, an average as low as 4.4 pounds per square inch was observed. The reason for this difference was that during Trial I, the pressure maintenance was difficult to keep and by the time Trial II was carried out, most of the problems that showed up in Trial I were remedied.

Table XII. Dry matter digestibility as affected by fermentation and additives

Additives	ALFALFA				CORN				SUDAN				SUDAX			
	P	Q	R	S	P	Q	R	S	P	Q	R	S	P	Q	R	S
							Before fermentation									
Trial I	17.7	32.0	29.8	28.1	23.2	24.0	22.8	24.1	14.1	20.0	17.2	15.3	5.1	6.5	7.5	4.8
Trial II	7.2	8.0	10.4	5.6	14.2	15.5	17.3	13.5	9.2	8.1	9.4	8.7	6.7	6.5	7.3	5.0
Ave.	12.5	20.0	20.1	16.8	18.7	20.0	20.0	18.8	11.7	14.0	13.3	12.0	5.9	6.5	7.4	5.0
							After fermentation									
Trial I	11.9	19.1	17.0	13.7	5.0	4.9	4.2	4.6	6.4	7.7	9.9	7.0	3.9	5.2	5.4	5.3
Trial II	12.7	12.3	10.9	12.1	5.0	4.9	5.3	7.3	6.9	5.6	6.8	7.1	4.7	3.4	4.8	3.9
Ave.	12.3^{c}	15.7^{b}	14.0^{b}	12.9^{c}	5.0^{b}	5.0^{b}	5.0^{b}	6.0^{b}	6.7^{c}	6.8^{c}	8.4^{b}	7.0^{bc}	4.3^{b}	4.3^{b}	5.2^{b}	4.6^{b}

b, c Means under each forage column bearing different superscript letters differ significantly ($P<.05$).

It was planned to collect run-off from the silos and determine how much dry matter was lost through this route. Due to an accident in the first trial, most of the run-off was lost. Hence, no figures were determined for this trial. However, the results of dry matter recovery in run-off for Trial II are presented in Table XIV. The results showed that the loss of dry matter through run-off ranged from .06 gm to .24 gm per c.c. Expressed as total percentage dry matter loss during fermentation, alfalfa was the highest with 1.67%, followed by sudan with .78% then corn and sudax with .56 and .50%, respectively. The high loss by alfalfa could possibly have been due to its high leafiness and succulence. Sudan coming next could have been because it was equally leafy and succulent. Consequently, the more matured and tougher sudax had the least loss through run-off. There was some relationship between dry matter loss through run-off and the moisture content of each forage. Among other things, the wetter the silage the more dry matter loss. Sudax with the least moisture (65.3%) lost the least dry matter through run-off whereas alfalfa (72.1%) lost more dry matter through run-off.

Simple correlation analyses were run between the variables. There were very few high correlations. Some were considered to be of some value. There was a high correlation between dry matter content determined prior and after fermentation (coefficient of 0.91). The production of acetic acid was negatively correlated (-0.38) to that of

butyric acid. This showed that acetic acid production was inversely related to the production of butyric acid. Another striking correlation was that between the results of the 12 hour post fermentation digestion and lactic acid production. The coefficient here was -0.58. As was mentioned while trying to rationalize the disparity between digestibility of fermented and unfermented silages, it shows here that the high lactic acid might have been lost during drying of the samples and, hence, the materials left were less digestible. Another high correlation of 0.86 was observed between the 24 hour post fermentation digestion and crude protein content. This seemed to be understandable since, as it was shown in Table VI, alfalfa with the highest protein content produced the least lactic acid which in turn was negatively correlated to digestibility.

Table XIII. Overall silo pressure

Forages:	Alfalfa				Corn				Sudan				Sudax				Ave.
Additives:	P	Q	R	S	P	Q	R	S	P	Q	R	S	P	Q	R	S	
Trial I:	8.6	8.6	9.1	10.0	6.3	7.8	6.7	6.8	4.4	7.1	7.1	8.5	9.3	9.3	8.6	8.0	7.8
Trial II:	9.5	9.5	9.4	6.1	9.7	9.7	9.7	9.7	9.8	9.8	9.9	9.5	8.8	9.9	10.0	10.0	9.5

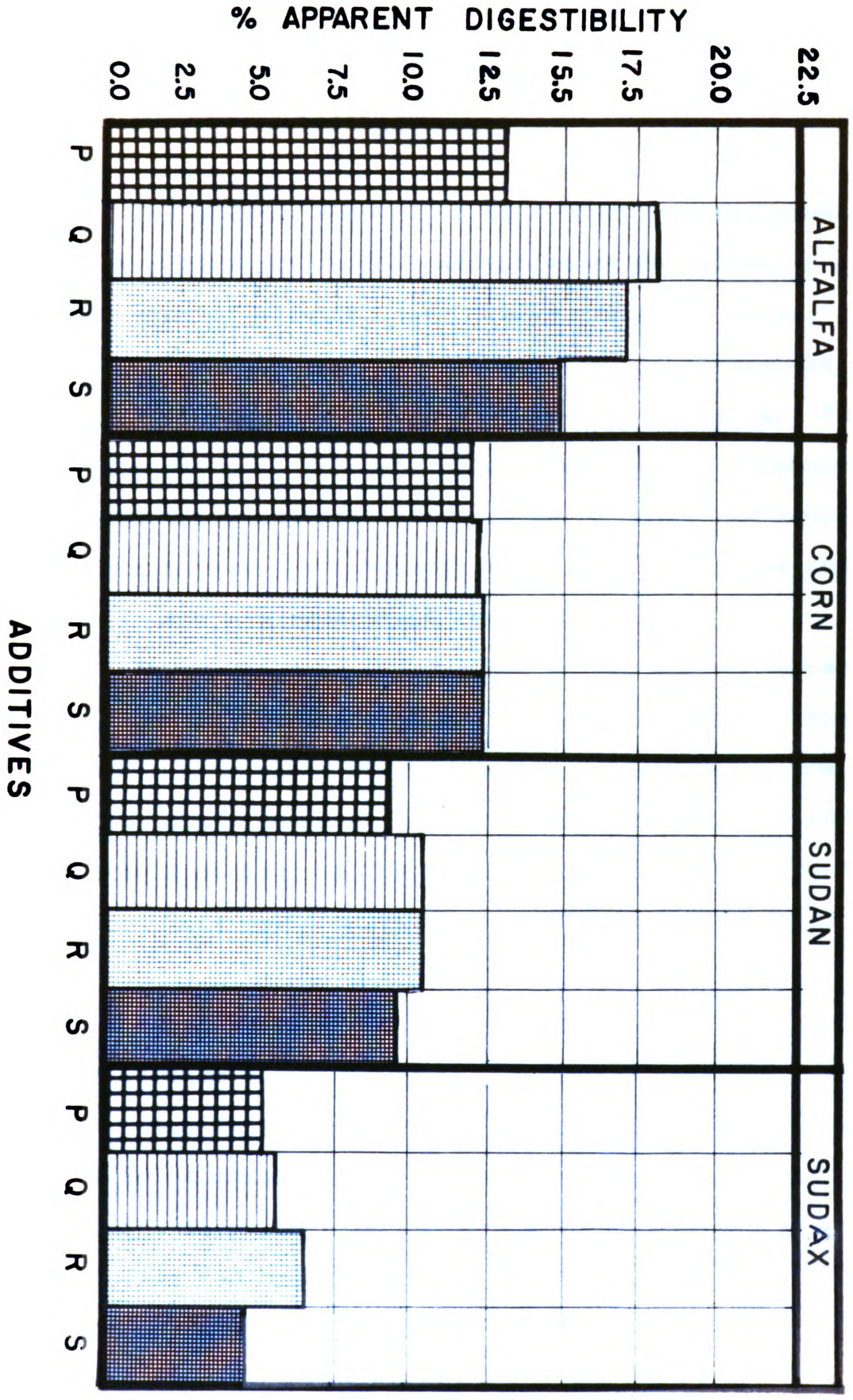


Fig. IV. : Pooled additive effect on dry matter digestibility of each silage; fermented and un-

DISCUSSION

The primary purpose of this study was to investigate the effects of various additives on fermentation of silages made of alfalfa, corn, sudan grass and sudax. For these findings to be useful for statistical inferences, it was necessary to work with as many silages as possible. This objective was met, in part, by the fact that the results presented above represented averages of duplicate samples from four forages each treated with three additives and one control; and the experiment replicated once.

There was some discrepancy in dry matter content of the silages in the two trials. Since there was no apparent reason for this, from the standpoint of procedure, the author felt that the difference might have been due to a higher lactic acid content in Trial II which was lost in the drying process. This could possibly lead to lower dry matter content.

Adding urea to the various silages resulted in higher pH than the other additives and the control. These results were in agreement with work reported by Schmutz (1962) and Klosterman *et al.* (1961). Considering the effect of forages on pH, the values for alfalfa were the highest. Sudan grass had the lowest pH. Surprisingly, this forage had the highest moisture content (about 79%). It could be

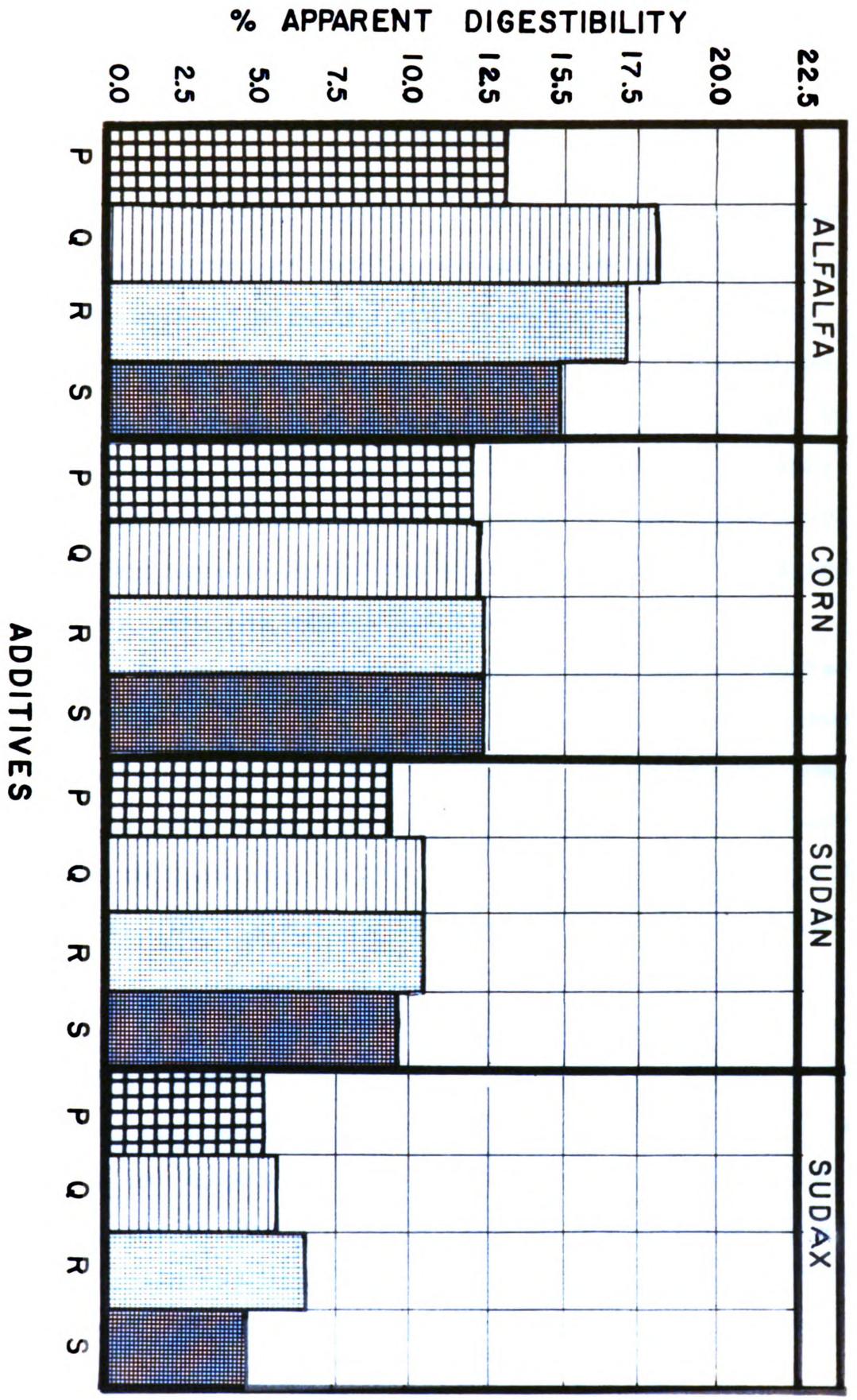


Fig. IV. : Pooled additive effect on dry matter digestibility of each silage; fermented and un-

DISCUSSION

The primary purpose of this study was to investigate the effects of various additives on fermentation of silages made of alfalfa, corn, sudan grass and sudax. For these findings to be useful for statistical inferences, it was necessary to work with as many silages as possible. This objective was met, in part, by the fact that the results presented above represented averages of duplicate samples from four forages each treated with three additives and one control; and the experiment replicated once.

There was some discrepancy in dry matter content of the silages in the two trials. Since there was no apparent reason for this, from the standpoint of procedure, the author felt that the difference might have been due to a higher lactic acid content in Trial II which was lost in the drying process. This could possibly lead to lower dry matter content.

Adding urea to the various silages resulted in higher pH than the other additives and the control. These results were in agreement with work reported by Schmutz (1962) and Klosterman et al. (1961). Considering the effect of forages on pH, the values for alfalfa were the highest. Sudan grass had the lowest pH. Surprisingly, this forage had the highest moisture content (about 79%). It could be

inferred that higher moisture content would promote acid production; hence, lower the pH. Klosterman et al. (1961) found increased acid (acetic and lactic) production corresponding to increased moisture content of high moisture ear corn.

The results on crude protein were also somewhat in agreement with the findings of Schmutz (1962) who found that high moisture corn silage treated with urea at 20 lb. a ton resulted in crude protein values of 13.6%. This was higher than the average of 10.8% protein in the urea treated corn silage found in the experiment reported here, where urea was added at the rate of 10 lb. per ton. Wise, et al. (1944) also found that urea treated corn silage had a crude protein content of 10.79%, whereas untreated silage had 7.48%. Bentley et al. (1955), and Labedinsky and Corb (1960) reported increases in crude protein with urea treated silages.

Apart from the unusually high acetic acid production of about 138.5 micromoles per gram of silage in alfalfa in Trial I (Table VII), the general production was well below 85 micromoles per gram. Taking acetic acid as a percent of total acids produced, this was found to be about 40%. Lactic acid was about 55% and propionic and butyric made up 5%. Schmutz (1962) observed that acetic could range from zero to 30% total acidity while Nicholson and Cunningham (1964) reported acetic acid to be 20.4%, lactic 79.6% and butyric 0% in untreated silages and 1% lime treated bromegrass

Table XII. Dry matter digestibility as affected by fermentation and additives

Additives	ALFALFA				CORN				SUDAN				SUDAX			
	P	Q	R	S	P	Q	R	S	P	Q	R	S	P	Q	R	S
							Before fermentation									
Trial I	17.7	32.0	29.8	28.1	23.2	24.0	22.8	24.1	14.1	20.0	17.2	15.3	5.1	6.5	7.5	4.8
Trial II	7.2	8.0	10.4	5.6	14.2	15.5	17.3	13.5	9.2	8.1	9.4	8.7	6.7	6.5	7.3	5.0
Ave.	12.5	20.0	20.1	16.8	18.7	20.0	20.0	18.8	11.7	14.0	13.3	12.0	5.9	6.5	7.4	5.0
							After fermentation									
Trial I	11.9	19.1	17.0	13.7	5.0	4.9	4.2	4.6	6.4	7.7	9.9	7.0	3.9	5.2	5.4	5.3
Trial II	12.7	12.3	10.9	12.1	5.0	4.9	5.3	7.3	6.9	5.6	6.8	7.1	4.7	3.4	4.8	3.9
Ave.	12.3^{c}	15.7^{b}	14.0^{b}	12.9^{c}	5.0^{b}	5.0^{b}	5.0^{b}	6.0^{b}	6.7^{c}	6.8^{c}	8.4^{b}	7.0^{bc}	4.3^{b}	4.3^{b}	5.2^{b}	4.6^{b}

b, c Means under each forage column bearing different superscript letters differ significantly ($P<.05$).

It was planned to collect run-off from the silos and determine how much dry matter was lost through this route. Due to an accident in the first trial, most of the run-off was lost. Hence, no figures were determined for this trial. However, the results of dry matter recovery in run-off for Trial II are presented in Table XIV. The results showed that the loss of dry matter through run-off ranged from .06 gm to .24 gm per c.c. Expressed as total percentage dry matter loss during fermentation, alfalfa was the highest with 1.67%, followed by sudan with .78% then corn and sudax with .56 and .50%, respectively. The high loss by alfalfa could possibly have been due to its high leafiness and succulence. Sudan coming next could have been because it was equally leafy and succulent. Consequently, the more matured and tougher sudax had the least loss through run-off. There was some relationship between dry matter loss through run-off and the moisture content of each forage. Among other things, the wetter the silage the more dry matter loss. Sudax with the least moisture (65.3%) lost the least dry matter through run-off whereas alfalfa (72.1%) lost more dry matter through run-off.

Simple correlation analyses were run between the variables. There were very few high correlations. Some were considered to be of some value. There was a high correlation between dry matter content determined prior and after fermentation (coefficient of 0.91). The production of acetic acid was negatively correlated (-0.38) to that of

butyric acid. This showed that acetic acid production was inversely related to the production of butyric acid. Another striking correlation was that between the results of the 12 hour post fermentation digestion and lactic acid production. The coefficient here was -0.58. As was mentioned while trying to rationalize the disparity between digestibility of fermented and unfermented silages, it shows here that the high lactic acid might have been lost during drying of the samples and, hence, the materials left were less digestible. Another high correlation of 0.86 was observed between the 24 hour post fermentation digestion and crude protein content. This seemed to be understandable since, as it was shown in Table VI, alfalfa with the highest protein content produced the least lactic acid which in turn was negatively correlated to digestibility.

Table XIII. Overall silo pressure

Forages:	Alfalfa				Corn				Sudan				Sudax				Ave.
Additives:	P	Q	R	S	P	Q	R	S	P	Q	R	S	P	Q	R	S	
Trial I:	8.6	8.6	9.1	10.0	6.3	7.8	6.7	6.8	4.4	7.1	7.1	8.5	9.3	9.3	8.6	8.0	7.8
Trial II:	9.5	9.5	9.4	6.1	9.7	9.7	9.7	9.7	9.8	9.8	9.9	9.5	8.8	9.9	10.0	10.0	9.5

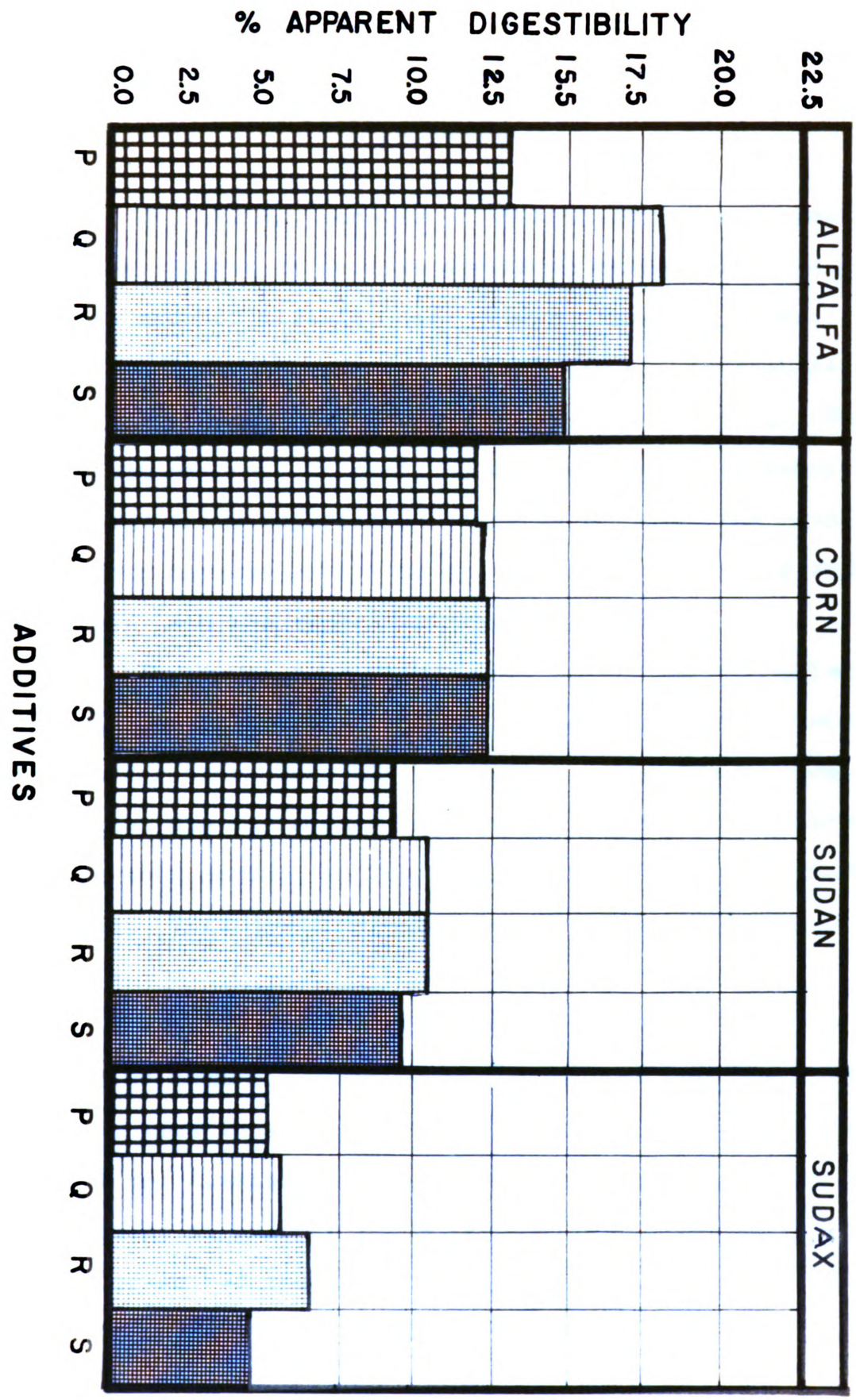


Fig. IV. : Pooled additive effect on dry matter digestibility of each silage; fermented and un-

DISCUSSION

The primary purpose of this study was to investigate the effects of various additives on fermentation of silages made of alfalfa, corn, sudan grass and sudax. For these findings to be useful for statistical inferences, it was necessary to work with as many silages as possible. This objective was met, in part, by the fact that the results presented above represented averages of duplicate samples from four forages each treated with three additives and one control; and the experiment replicated once.

There was some discrepancy in dry matter content of the silages in the two trials. Since there was no apparent reason for this, from the standpoint of procedure, the author felt that the difference might have been due to a higher lactic acid content in Trial II which was lost in the drying process. This could possibly lead to lower dry matter content.

Adding urea to the various silages resulted in higher pH than the other additives and the control. These results were in agreement with work reported by Schmutz (1962) and Klosterman et al. (1961). Considering the effect of forages on pH, the values for alfalfa were the highest. Sudan grass had the lowest pH. Surprisingly, this forage had the highest moisture content (about 79%). It could be

inferred that higher moisture content would promote acid production; hence, lower the pH. Klosterman et al. (1961) found increased acid (acetic and lactic) production corresponding to increased moisture content of high moisture ear corn.

The results on crude protein were also somewhat in agreement with the findings of Schmutz (1962) who found that high moisture corn silage treated with urea at 20 lb. a ton resulted in crude protein values of 13.6%. This was higher than the average of 10.8% protein in the urea treated corn silage found in the experiment reported here, where urea was added at the rate of 10 lb. per ton. Wise, et al. (1944) also found that urea treated corn silage had a crude protein content of 10.79%, whereas untreated silage had 7.48%. Bentley et al. (1955), and Labedinsky and Corb (1960) reported increases in crude protein with urea treated silages.

Apart from the unusually high acetic acid production of about 138.5 micromoles per gram of silage in alfalfa in Trial I (Table VII), the general production was well below 85 micromoles per gram. Taking acetic acid as a percent of total acids produced, this was found to be about 40%. Lactic acid was about 55% and propionic and butyric made up 5%. Schmutz (1962) observed that acetic could range from zero to 30% total acidity while Nicholson and Cunningham (1964) reported acetic acid to be 20.4%, lactic 79.6% and butyric 0% in untreated silages and 1% lime treated bromegrass

silage showed 26.1% acetic, 55.3% lactic and 18.6% butyric. In this last reference, butyric acid content seems too high but this could have been due to the conditions of aeration of the silages. There was a difference in the amounts of acetic acid produced in the two trials. No apparent explanation could be given either for the high values for alfalfa in Trial I or the overall difference between the two trials. More research may be recommended in this area to find out whether these discrepancies were due to factors like differences in moisture content, or some other unidentified variables.

Very few workers have reported on the production of propionic acid in silages. In this study, there was no consistent pattern for this acid. It was seen that some forages that produced most propionic in Trial I produced practically none in Trial II. However, sudax was consistently low in both. It could also be said here that as researchers continue to seek more precision in silage fermentation product investigation methods, more attention may be directed towards this minor acid rather than towards the oftbeaten tract of the major fatty acids, or lactic and acetic acids.

The overall average lactic acid production was about 200 micromoles per gram of silage material on a dry basis. This compares favorably with 198 micromoles found by Schmutz (1962) and results found by Klosterman *et al.* (1961), and also by Nicholson and Cunningham (1964). Klosterman showed that there was a corresponding increase in both lactic and

acetic to increase in moisture. If further research should confirm these findings then there could be a hopeful future for high moisture corn silage and haylages in ruminant nutrition.

The second experiment was to obtain some digestibility data on dry matter as measured by its disappearance in the rumen of a fistulated steer. The results showed that unfermented silages were more digestible than the fermented ones. This finding seemed to go contrary to common sense. Since digestion is essentially a process of fermentation, one would expect a partially fermented silage to be on its way to easy digestion and hence would show more dry matter disappearance. As already mentioned under the presentation of the results, this low percentage digestion could have been due to drying of the samples whereby the already broken down constituents, like the fatty acids, were driven off by heat and the material left was therefore less digestible.

More work may be recommended in this area. In the event that such a work is to be done, it may be desirable to carry it out in two phases. More could be done in the same way as was carried out in this study to see if these observations would be confirmed. Some means should be found whereby differences in weights as a result of digestion could be detected without subjecting the silages to drying at all, or not to dry them to the same extent as was done in this study.

On the basis of the results from the present study, it would be futile to draw any conclusions as to which additive was the best. Since the Bio-zyme ("P") and the Silo Guard ("Q") are rather recent commercial additives, it would seem necessary to investigate their silage improving potentials by carrying out more research. Nevertheless, some additive differences showed up. Figure IV shows the pooled effect of the additives on all forages in the two trials. In essence, this figure shows that during fermentation of alfalfa, Silo Guard and urea increased digestion of dry matter over the control, while Bio-zyme actually decreased dry matter digestibility. Differences between additives for the other forages were small.

Losses in dry matter as determined from silage run-off are presented in Table XIV. These losses ranged from .44% to 1.98% with the greatest loss occurring in alfalfa, followed by sudan and then corn and sudax.

The high rate of dry matter losses in both alfalfa and sudan could be related to the high leafiness and perhaps high moisture content of these forages. Corn and sudax, though grainy, had high cellulose content due to their characteristic stems and advanced stage of maturity.

Except in sudan where the additive R (urea) had up to 1.07% dry matter loss, additive effect on each individual forage was well within the same range or they did not differ very much. The results from this study have fallen below

those obtained by Schmutz (1962). Schmutz reported dry matter loss in high moisture ear corn silage to be about 5%. However his silage was fermented for periods ranging up to 12 months.

SUMMARY

During the winter and the early part of Spring 1965, alfalfa, center-cut corn, sudan grass and sudax were treated with feed grade urea (42%N) and two commercial silage additives and ensiled in miniature galvanized metal silos for six days periods. Air tight conditions and an average pressure 8.7 lb. per square inch were maintained, thus similating, as far as possible, conditions in conventional silos. The experiment consisted of one run and one replicate. The ensiled forages were sampled before and again after fermentation and the samples were used for chemical as well as dry matter determinations. Digestion trials were also carried out on these samples in the rumen of a fistulated Holstein steer.

Chemical analyses were done on dry matter, pH, crude protein, and volatile fatty acids. The digestion trials were carried out on the dry matter using a modified form of the nylon bag technique in the rumen of a fistulated Holstein steer. Of all the factors studied, the pH both before and after fermentation had the smallest standard deviation. Every other factor varied greatly.

The additives used had no significant affect on dry matter in the various silages, but adding urea resulted in slightly higher dry matter than the control and other additives. There was more dry matter (ave. 35.49%) conserved in Trial I than in II (26.94%).

The average pH before fermentation was 5.4 and after fermentation, this was depressed to 3.7. Silages treated with urea were slightly higher in pH (4.0) and this additive was more consistent in its effect on pH than the rest.

The effect of additive on crude protein was significant ($P < .05$). Urea treated silages consistently resulted in significantly higher crude protein content while the other additives apparently had no influence on crude protein during fermentation.

As regards organic acid production in the entire fermentation experiment, lactic was produced in the greatest amount followed by acetic, propionic and butyric. There was more acetic acid produced in Trial I than in II and adding urea resulted in significantly ($P < .05$) higher acetic acid production in both trials. The other additives did not significantly affect acetic acid production.

Propionic acid production was both small and erratic whereas that of butyric acid was only negligible.

Addition of urea resulted in significantly ($P < .05$) higher lactic acid production in both trials; the levels being 195.6 and 220 micromoles per gram of dry forage in Trials I and II, respectively. The other additives did not significantly affect lactic acid production during fermentation. Average lactic acid production was higher in Trial II than I.

The overall forage effect on acid production was significant ($P < .05$) in both trials. In Trial I, acetic acid

production by alfalfa was highly significant ($P<.001$). This forage showed the least lactic acid production and the overall average of lactic acid for sudan grass was the highest.

In the digestibility trial, additive and forage effect and the interaction between both were significant ($P<.01$) for the first 12 hours and ($P<.1$) for the 24 hour period. The effect of time interval was outstanding. There was a steady progression in digestion through time in that the digested amounts approximately doubled for every additional 12 hour period. This direct proportion was true especially for the unfermented materials.

It was observed that the unfermented material showed more dry matter digestion than the fermented ones. There was more dry matter disappearance in Trial I than in II. Because of maturity and species differences in the forages no attempt was made to compare the forage effect on dry matter digestion. An average pressure of 7.9 and 9.5 pounds per square inch were maintained in Trials I and II respectively, during fermentation. Run-off was collected from Trial II and dry matter loss was determined. The average loss range from .6 to 2.4 grams per 100 ml run off. Simple correlations analysis showed high correlations between dry matter determinations prior to and after fermentation (0.91); 24 hour post fermentation and crude protein (0.84). There were negative correlations (coeff. -0.38) observed between butyric and acetic acids, and (coeff. -0.58) between the 12 hour post fermentation period and lactic acid.

BIBLIOGRAPHY

A.O.A.C. 1961. Official methods of analysis of the Association of Official Agricultural Chemists. 9th edition, Association of Official Agricultural Chemists, Publishers, Washington 4, D.C.

Allen, L.A., Harrison, J., Watson, S.J., and W.S. Ferguson. 1937. A study of the chemical and bacteriological changes occurring in grass silage. Jour. Agric. Sci. 27:271-293.

Archibald, J.G., Barnes, H.D., Fennar, H., and B. Gersten. 1962. Digestibility of alfalfa hay and canary grass hay measured by two procedures, Jour. Dairy Sci. 45:858-60.

Arttwin, Ilmari Virtanen. 1938. Cattle fodder and human nutrition with special reference to biological nitrogen fixation. Cambridge Univ. Press.

Barker, S.B. and W.B. Summerson. 1941. The colorimetric determination of lactic acid in biological material. Jour. Biol. Chem. 138:535.

Barnett, A.J. 1954. "Silage Fermentation," Academic Press Inc., New York 10, N.Y.

Baumgardt, R.B., Cason, J.L., and M.W. Taylor. 1962. Simplified artificial rumen procedure for obtaining repeatable estimates of forage nutritive value, Jour. Dairy Sci., 45:62-68.

_____, _____, and _____. 1962b. Evaluation of forages in the laboratory, Jour. Dairy Sci. 45:59-61.

Bender, C.B., Bosshardt, D.K., and O.F. Crawett. 1941. "Some chemical changes occurring in molasses grass silage," Jour. Dairy Sci. 24:147-51.

Benne, E.J. and M. Wacasey. 1960. The chemistry and biology of silage, Mich. Agric. Ext. Folder F-290.

Bentley, O.G., Klosterman, E.W., and P. Engle. 1955. The use of urea to increase the crude protein content of corn silage for fattening steers. Ohio Agric. Exp. Sta. Res. Bul. 960.

Bowden, J.M., and D.C. Church. 1962. Artificial rumen investigation, I. varitability of dry matter and cellulose digestibility and production of volatile fatty acids, Jour. Dairy Sci. 45:972-79.

Brattzler, R.L., Cowan and R.W. Swift. 1956. Grass silage preservation with sodium metabiosulfite. Jour. An. Sci. 15:1, 163-170.

Cabell, C.A., Davis, R.E., and R.A. Saul. 1962. "High moisture grain stored in different type silos then compared by nutritive values." (Abstract) Jour. Agric. Sci. 21:141.

Clark, John D. 1961. Use of the nylon bag technique in measuring digestibility. Dept. Animal Sci., Washington, State Univ. (Personal communication).

Cline, T.R., Coarvigus, U.S., Hatfield, E.E., Forbes, R.M., and B.B. Doane. 1960. "Metabolism study of ensiled high-moisture ground ear corn, ensiled high-moisture ground ear corn, ensiled high-moisture shelled corn and field dried shelled corn with lambs. Illinois Sheep Day Report, pp. 1-3.

Colovos, N.F., Keener, H.A. and H.A. Davis. 1958. Effect of pulverized limestone and dicalcium phosphate on the nutritive value of dairy cattle feed. Jour. Dairy Sci. 41:626-682.

Crampton, E.W., and L.A. Maynard. 1938. The relationship between cellulose and lignin content to the nutritive value of animal feeds. Jour. of Nutrition 15:383.

Cullison, A.E. 1944. "The use of urea in making silage from sweet sorghum," Jour. Agric. Sci. 3:59-62.

Davis, G.K., Becker, R.B., Dix, Arnold P.T., Connar, C.L., and S.P. Marshall. 1944. "Urea in sorghum silage," (Abstract) Jour. Dairy Sci. 27:649.

Dobrogosz, and R.W. Stone. 1957. Microbiological and chemical studies on metabisulfate treated alfalfa silage, Bacterial Proc. p. 23.

Dyer, I.A. 1961. Urea in cattle feeding rations. Feed Agr. 11(9): 29-31.

Esten, W.M., and C.J. Mason. 1912. "Silage Fermentation," Storrs Agric. Expt. Sta. Bul. 70.

Erwin, E.S., and K.G. Elliston (Abstract) 1959. Rapid method of determining digestibility of concentrates and roughages in cattle. Jour. An. Sci. 18:1518.

Ferguson, W.S., and S.J. Watson. 1937. Losses of dry matter and digestible nutrients in low temperature silage with and without added molasses or mineral acids. Jour. Agric. Sci. 27:67.

Fina, L.R. 1958. Artificial techniques for studying rumen digestion in vivo, Jour. Agric. Sci. 17:667-74.

Gallup, W.D., Pope, L.S., and C.K. Whitehair. 1953a. Urea in rations for cattle and sheep: A summary of expt. station 1944-1952. Okla. Agric. Expt. Sta. Bul. B-409.

_____, _____, and _____. 1953b. Urea as a source of protein in livestock rations. Okla. Expt. Sta. Circular C-137.

Gordon, C.H., Derbyshire, J.C., Wiseman, H.G., and E.A. Kane. 1961. Preservation and feeding value of alfalfa stored as hay, haylage and direct-cut silage. Jour. Dairy Sci. 44:1299-1311.

Hall, H.H., Etchells, J.L., Jones, I.D., and W.M. Lewis. 1954. Microbiological and chemical studies of sweet potato vine silage, Jour. Dairy Sci. 37:1325-1336.

Hansen, E.L., Mitchell, G.E., Hatfield, E.E., Jensen, A.H. and K.E. Harshberger. 1959. Harvesting, ensiling, feeding high-moisture shelled corn, Illinois Research, pp. 305.

Harris, L.E., and H.H. Mitchell. 1941a. The value of urea in the synthesis in the paunch of the ruminant. I. In maintenance Jour. Nutrition, 22:167-182.

_____, and _____. 1941b. The value of urea in the synthesis in the paunch of the ruminant. II. Growth, Jour. Nutrition 22:183-96.

Haris, L.E., Work, S.H., and L.A. Henke. 1943. The utilization and soybean oil meal by Steers, Jour. Agric. Sci. 2:328-335.

Hayden, C.C., Perkins, A.E., Monroe, C.F., Krauss, W.E., Washburn, R.G., and C.E. Knoop. 1945. A summary of 10 yrs. work. Ohio Agric. Expt. Sta. Bul. 656.

Herman, N.A., Ragsdale, A.C., and W. Heathman. 1941. Trench silos for preserving cereals treated with molasses or phosphuric acid. Jour. Dairy Sci. 24:514-15.

Hopson, J.D. 1961a. (Abstract) Use of the nylon bag technique in in vivo cellulose digestion in forage evaluation. Jour. Agric. Sci. 20:960.

Hopson, D.J., and R.R. Johnson. 1961. Use of the dacron bag technique in in vivo cellulose digestion in forage evaluation. Jour. An. Sci. 20:960.

Hunter, O.W. 1917. Microorganism and heat production in silage fermentation, Jour. Agr. Res. 10:75-83.

Ingham, R.W., W.A. King, W.C. Russel and C.B. Bender. 1949. Grass silage and dairying. Rutgers University Press, New Brunswick.

Irwin, H.M., Langstow, C.W., and C.H. Gordon. 1956. Development Organic Acids in Silage, Jour. Dairy Sci. 39:940.

Kempton, A.G., and C.E. San Clemente. 1959. Chemistry and microbiology of forage-crop silage, Appl. Microbiol. 7:362-67.

Klosterman, E.W., Johnston, R.R., Scott, H.W., Maxon, A.L., and J. Van Stavern. 1960a. Whole plant and ground ear corn silage, their acid contents. Feeding value and digestibility, Jour. Agric. Sci. 19:522-32.

_____, _____, _____, _____, and H. W. Scott. 1960b. Feeding value of treated and untreated corn silage and dry ear corn for fattening cattle. Beef Cattle Research, Ohio Agric. Expt. Sta. Animal Science Series, 21:9-18.

_____, Moxon, A.L., Johnston, R.R., Scott, H.W., and J. Van Stavern. 1961. Feeding value for fattening cattle of corn silages treated to increase their content of organic acids. Jour. Agric. Sci. 20:493-96.

Krause, B. and R.H. Hopkins. 1937. Biochemistry applied to malting and brewing, D. Van Nostrand Co. Inc., New York.

Kronlik, J.T., Burkey, L.A., Gordon and C.H. Wiseman. 1955. Microbial population of the green plants and of the cut forage prior to ensiling. Jour. Dairy Sci. 38: 256-272.

Langston, C.W., Irwin, H., Gordon, C.H., Bouma, C., Wiseman, H.G., Melin, C.G., and L.A. Moore. 1958. Microbiology and chemistry of grass silage, United States Dept., Agric. Bul. 1187.

_____, and C. Bouma. 1960a. A study of the microorganisms from grass silage. II. The lactobacilli. Appl. Microbiol. 8:223-34.

_____, and _____. 1960b. Types and sequence changes of bacteria in orchard grass and alfalfa silages. Jour. Dairy Sci. 43:1575-84.

Langston, C.W., Wiseman, H.G., Gordon, C.H., Jacobson, W.C., Melin, C.G., Moore, L.A., and J.R. McCalment. 1962. Chemical and bacteriological changes in grass silage during the early stages of fermentation. I. Chemical changes, Jour. Dairy Sci. 45:396-402.

Lusk, J.W., Browning, C.B., and J.T. Miles. 1962. Small sample *in vivo* cellulose digestion procedure for forage evaluation. Jour. Dairy Sci. 45:69-73.

Matrone, G.A. 1955. A study of lignin and cellulose methods for the chemical evaluation of feeds, M.S. thesis, Cornell University, Ithaca, New York.

Means, R.H. 1945. Urea added to sorghum silage increases value, Miss. Farm Res. 8:7.

Nicholson, J.W.G. and H.M. Cunningham. 1964. Addition of limestone to immature corn and grass silages. Jour. An. Sci. 23:1072-1078,

Pettyjohn, J.D., J.M. Leatheweed. 1964. Simplified technique for *in vitro* comparison of cellulose digestibility and dry matter digestibility of forages. Jour. Dairy Sci. 47:

Reid, J.T. 1953. Urea as a protein replacement for ruminants. A Review, Jour. Dairy Sci. 36:955-96.

_____. 1954. Protein replacements for ruminants. 46th Ann. Conv. of Am. Feed. Manf. Assn.

Russell, E.J. 1908. The chemical changes taking place during the ensilage of maize. Jour. Agri. Sci. 2:392-410.

Salsbury, R.L., and A.L. Vanderkolt. 1958. The rates of digestion of cellulose of some plant fractions by rumen microorganism *in vitro*, Jour. Agric. Sci. 17: 293-297.

Scheffé, H. The Analysis of Variance. 1959. John Wiley & Sons, Inc., New York.

Schmutz, W.G. 1962. The chemistry, bacteriology and nutritive value of ensiled high-moisture ground-ear corn. M.S. thesis, Michigan State University, 1962.

Sherman, J.M. and S.I. Bechdel. 1918. Corn Stover Silage, J. Agric. Res. 12:589-600.

Snedecor, G.W. 1959. Statistical Methods, 5th edition, The Iowa State College Press, Ames, Iowa.

Stewart, W.E., and L.H. Schultz. 1961. *In vitro* volatile fatty acids production from various feed by bovine rumen microorganisms. Jour. of An. Sci. 21:360-365.

Stone, R.W., Bechdel, S.I., McAuliff, H.D., Murdock, F.R., and R.C. Malzahn. 1943. The fermentation of alfalfa silage, Pa. Agric. Expt. Sta. Bul. 444.

Thomas, W.E., Loosh, J.K., Williams, H.H. and L.A. Maynard. 1951. The utilization of inorganic sulfates and urea nitrogen by lambs. Jour. Nutrition 43:515.

Todd, J.R. 1956. Investigation into the chemical composition and nutritive value of certain forage plants at medium altitudes in the tropics. Jour. Agric. Sci. 47:35-39.

Van Keuren, R.W., Heinemann, W.W., Hopson, J.D. and R.R. Johnson. 1962. Study for a nylon bag technique for in vivo estimation of forage digestibility, Jour. Agr. Sci. 21:340-45.

Virtanen, A.J. 1938. Cattle fodder and human nutrition with special reference to biological nitrogen fixation. Cambridge University Press.

Walker, D.M., and W.R. Hepburn. 1956. I. The nutritive value of roughages for sheep. II. The relationship between the grass digestible energy and chemical composition of silages. Jour. Agri. Sci. 47:172-86.

Watson, S.J. 1939. The science and practice of conservation: grass and forage crops. Vol. I. McMillan Co., London, p. 265.

Wise, G.H., Mitchell, J.H., LaMaster, J.P. and D.B. Roderick. 1944. Urea treated corn silage vs. untreated corn silage as a feed for lactating dairy cows. (Abstract) Jour. Dairy Sci. 27:649.

Wiseman, H.G., and H.W. Irwin. 1957. Determination of organic acids in silage. Agricultural and Food Chemistry 5: 213-15.

Woll, 1939. Modern silage making, food and life, Yearbook of Agriculture, U.S.D.A.

Woodward, T.E., and J.B. Shepherd. 1944. Corn silage made with the addition of urea and its feeding value (Abstract). Jour. Dairy Sci. 27:648.

Yang, M.G. and J.W. Thomas. 1962. A study of dry matter disappearance in nylon bags suspended in rumen as affected by heat, time, and rations and its use in forage evaluation. (Mich. State University Dairy Sci. Monograph).