

AN ABSTRACT OF THE THESIS OF

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Title: THE EFFECT OF VARIOUS ADDITIVES ON NUTRITIVE  
VALUE OF RYEGRASS STRAW SILAGE

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Observations were made on silage pH, chemical composition and 24 hr in vitro dry matter digestion (IVDMD) to evaluate effects of various additives ensiled with annual ryegrass straw. Additives were compared in factorial designed trials utilizing .95 l glass jar silos. Selection of additives used in each subsequent trial was based on results of preceding trials and then combined with other additives. All additives were used as a percent of straw on a dry matter basis and incorporated with water to give 22% dry matter in silages.

Adding 4.5% NaOH:KOH allowed greater ( $P < .01$ ) silage IVDMD than .5 g cellulase while this gave more ( $P < .01$ ) IVDMD than straw ensiled with water. Including 10% and 20% molasses lowered ( $P < .01$ ) silage pH to more desirable levels, with or without the other additives. Using 20% molasses resulted in more favorable silage aspects and the lowest ( $P < .01$ ) acid detergent fiber

(ADF), acid detergent lignin (ADL) and cell wall constituents (CWC) for the silage.

Adding 1% urea to silage having 20% molasses or 4.5% hydroxide and 20% molasses increased ( $P < .01$ ) crude protein content and IVDMD. Adding 1.2% biuret to these silages raised ( $P < .01$ ) crude protein but did not affect IVDMD. Including .1% elemental sulfur raised ( $P < .01$ ) silage pH and a strong sulfur odor was present. Some spoilage occurred when sulfur was added to hydroxide-treated ensilage and was more evident in material containing urea. Reduced ( $P < .01$ ) ADF and CWC values were observed in hydroxide silage having sulfur-biuret additives.

Silage with 20% molasses and 1% urea had a lower ( $P < .01$ ) pH at 40 days than at 20 days ensilation. Adding .5% limestone lowered ( $P < .01$ ) pH further and improved ( $P < .01$ ) IVDMD at 40 days ensilation. Ensilage having 4.5% hydroxide and the same molasses and urea additives resulted in a more elevated ( $P < .01$ ) pH at 40 days. The addition of limestone to these additives lowered ( $P < .01$ ) silage pH while IVDMD was unaltered. Using .5% formic acid in these silages lowered ( $P < .01$ ) pH while not affecting IVDMD. All silages had less ( $P < .01$ ) residual ash at 40 days than at 20 days ensilation.

Ensiling straw with 20% molasses, 1% urea and .5% limestone gave lower ( $P < .01$ ) pH values than for hydroxide-treated silage

having the other additives at 0, 2, 4, 8, 16, 32 and 64 days ensilation. Decreased ( $P < .01$ ) ADF and CWC and increased ( $P < .01$ ) ADL values occurred in treated silage several days earlier ( $P < .01$ ) than untreated ensilage. Less ( $P < .01$ ) residual ash remained in either type silage after 32 days ensiling.

Formation of organic acids during the 0-64 days ensiling by hydroxide-treated material gave peak ( $P < .01$ ) lactic acid at 16 days then dropped ( $P < .01$ ) while acetic plateaued ( $P < .01$ ) at 16 days and butyric rose ( $P < .01$ ) sharply after 16 days. In contrast, untreated silage had a steady rise ( $P < .01$ ) of lactic to 64 days, acetic peaked ( $P < .01$ ) at 32 days, while butyric rose ( $P < .01$ ) at 8 days and declined at 32 days. Propionic acid was not detectable in untreated silage after 2 days ensilation while rises ( $P < .01$ ) occurred at 2 and 32 days in treated material.

IVDMD was higher ( $P < .01$ ) with 2-64 day substrate from treated silage while digestion for untreated was increased after substrate had been ensiled for 32 days. In vitro rumen liquor following 24 hr fermentation of hydroxide-treated silage allowed greater ( $P < .01$ ) concentrations of acetate and butyrate than untreated silage while numerical differences for propionate were not significant.

Lambs consumed more ( $P < .05$ ) dry matter and nitrogen from silage prepared in plastic barrels with 4.5% NaOH:KOH, 20%

molasses, 1% urea and .5% limestone as compared to ensilage having these additives except hydroxide. Material ensiled with hydroxide resulted in higher ( $P < .05$ ) digestibility of dry and organic matter, ADF, ADL, CWC and crude protein. Also more ( $P < .05$ ) nitrogen was retained while urine volume and density was greater numerically. Rumen fluid of these lambs contained more ( $P < .05$ ) acetic and propionic acids while butyric acid and  $C_2:C_3$  ratio were not significantly different.

Body weight gains during a 98 day trial were similar in heifers fed ryegrass silage, or straw ensiled with the molasses, urea, limestone and hydroxide additives, or the same straw silage without hydroxide when animals each received .9 kg barley-cottonseed meal supplement daily. Supplementing these silages with an equal amount of fat-molasses-urea mixture reduced ( $P < .05$ ) gains and silage intake was depressed. An additional daily .9 kg grain supplement during the final 38 days of the trial stimulated intake of either type straw silage while consumption of ryegrass ensilage was lowered. Animals consumed the straw silage having hydroxide more readily than the untreated straw ensilage throughout the trial. Dry matter of these silages were 37 and 40%, respectively, and little spoilage was evident following 60 days ensilation in plastic lined trench silos.

The Effect of Various Additives on Nutritive Value  
of Ryegrass Straw Silage

by

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DEDICATION

To my wife, ELENA,

and our children, Bernie, Christina and Michael,

I dedicate this thesis.

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# THE EFFECT OF VARIOUS ADDITIVES ON NUTRITIVE VALUE OF RYEGRASS STRAW SILAGE

## INTRODUCTION

Low nutritive value fibrous residues from industrial and agricultural sources has received considerable interest in recent years for use in cattle rations. These materials have been previously used for ruminant feeds when circumstances dictated, as in Europe during World War II.

Basically, there are two reasons for present day interest in these roughages. The competition between mono- and poly-gastrics for feedstuffs is intensifying and, in some parts of the world, the problem is acute. The unique ability of ruminants to convert these fibrous feedstuffs into milk and meat to provide animal protein for human consumption would obviously be beneficial. Secondly, disposal of these fibrous residues by the most convenient means, burning, has aggravated air pollution problems in some areas. The Willamette Valley is a case in point. Inclusion of this material in ruminant feeds would be one useful alternative.

The bulky nature and low digestibility of a material, such as straw, limits its feed value. Various methods exist to reduce the particle size and increase consumption. Also, several chemical

treatments are available to improve digestion (Donefer, 1972; Pidgen and Bender, 1972). An extensive evaluation of chemical treatments for ryegrass straw has demonstrated marked increases in digestibility (Anderson, 1972). Chemical residues in the feedstuff can reduce intake and rinsing out this residue may remove soluble nutrients. Neutralizing the chemically-treated materials has not been consistently beneficial to justify added cost. A practical means of overcoming the problem has been by either mixing the material with a mildly acidic feedstuff such as silage or ensiling and allowing gradual neutralization to occur (Lampila, 1963; Wilson and Pidgen, 1964).

Recent reports indicate that ensiling such additives as urea and sodium hydroxide with crop residues can promote improved animal performance when compared to untreated material (Koers, Klopfenstein and Woods, 1969; Koers, Woods and Klopfenstein, 1970; Colenbrander et al., 1971; Koers, Prokop and Klopfenstein, 1972). Other additives have resulted in less desirable ensilage and reduced intake or caused other practical limitations (Mowat, 1971; Yu Yu, Thomas and Emery, 1971; Goering et al., 1973).

Considerable attention has been given to the effects of various additives during ensilation of corn, legumes, or grasses, while relatively little information exists regarding the influence these additives may have when ensiled with low-quality roughage such as

ryegrass straw. The objectives of this study were:

1. Observe the effects of certain additives upon straw silage, pH, chemical composition and digestion.
2. Determine the changes in silage organic acids formed due to additives used and ensilation time.
3. Evaluate rumen fermentation, animal metabolism and performance as related to characteristics of straw silage having selected additives.

## LITERATURE REVIEW

By definition, silage is a feedstuff stored at a moisture level allowing anaerobic fermentation to occur. The term, silage additive, has been used to describe any number of materials incorporated into silages to prevent or alter fermentation processes, increase nutrient content, enhance silage preservation and improve palatability. Additives are sometimes referred to as preservatives or conditioners and innumerable single and combined additive preparations are on the market today.

### Selection of Additives

There are many factors which may determine the type and amount of additive to be used in silages. Foremost consideration must be given to the characteristics of the plant material to be ensiled. Moisture, content, proportion of soluble and structural carbohydrates, protein and minerals present, and buffering capacity of a given roughage material all will influence silage fermentation. Establishment and maintenance of anaerobic conditions, plus length and type of fermentation pattern all can affect silage quality. The pH, organic acids produced, and animal acceptance of the silage must also be considered.

The nearly infinite number of possible combinations of the



aforementioned variables make it necessary to limit the scope of this review to the needs for and the use of additives in relation to the ensiling of highly lignified plant residues. Additives will be considered regarding the effects upon major nutrients in the silage and consequent characteristics which may affect feed value for ruminants.

### Moisture and Soluble Carbohydrates

Straw, corn stalks, seed hulls, and other coarse textured materials generally require some degree of rehydration for silage making. The addition of water or other fluids serves to exclude air and aid in packing materials high in dry matter. Liquid media can also serve to more evenly distribute various additives throughout the ensilage (Barnett, 1954; Wieringa, 1960). Rehydration to a 50% silage dry matter content has been used with corn stover, corn cob, or straw materials (Kormscikov, 1968; Krause, Koers and Klopfenstein, 1968; Klopfenstein et al., 1972), while others have considered it necessary to add more moisture to reach a 30% and 20% silage dry matter content in similar material (Lampila, 1963; Goering et al., 1973). Variation has been attributed to simultaneous inclusion of other additives and the physical nature of the roughage. These fibrous materials can be considered an additive when used to absorb excess fluids and reduce seepage losses during ensilation

of high moisture forages (Barnett, 1954; Chhibbar and Singh, 1971; O'Donovan, Chen and Lee, 1972).

The presence of soluble carbohydrates will influence the rate, amount and type of organic acids formed during ensilation. The change in silage pH will alter the anaerobic bacterial populations and subsequently affect the preservation of ensilage quality (Dijkstra, 1954; Langston et al., 1962; Langston, Bouma and Conner, 1962). Molasses and other carbohydrates have been used extensively in silage making (Johnson et al., 1940; Axelsson, 1952; Rydin, Nilsson and Toth, 1956). Carbohydrates in highly lignified roughages are not readily available for a desirable silage fermentation, thus the addition of some form of soluble carbohydrate or an additive which may partially solubilize the structural plant carbohydrates is needed. Adding molasses to mature grass silage and ground barley to poplar bark ensilage has been of some benefit (Barker and Levitt, 1969; Enzman, Goodrich and Meiske, 1969; Robertson, Beacom and Shiels, 1971). Molasses has been used preferably when ensiling crop residue materials because of costs, the relative ease and uniformity of application when diluted, and as a convenient carrier for other additives. The amounts of molasses used have varied from 5 to 20%; an optimum level appears to be dependent on the roughage and other additives ensiled in a given situation (Sutoh and Uchida, 1969; O'Donovan, Chen and Lee, 1972).

### Plant Cell Wall Carbohydrates

The ensiling of straw, corn stover and cobs or other crop residues, with sufficient water to permit fermentation, has not appreciably improved feed value over the non-ensiled materials (Krause, Klopfenstein and Woods, 1968; Klopfenstein et al., 1972; Goering et al., 1973). The addition of chlorine, chloride and chlorite compounds have improved in vitro digestion following the ensilation period (Yu Yu, Thomas and Emery, 1970; Goering et al., 1973). Specific attack upon cell wall lignin was cited for the observed increases in digestibility. The usefulness of these silages was limited in subsequent in vivo trials by reduced intake (Yu Yu, Thomas and Emery, 1971; Goering et al., 1973). Chemical residues as well as mixing and safety problems were indicated as limitations to the practical use of these additives.

Improved in vitro and in vivo digestion of crop residue silages has been demonstrated by including hydroxides at ensiling time. The addition of 6% sodium hydroxide on a dry matter basis to corn stover and barley straw silages increased digestion; however, resultant silage pH was 7-7.5 and intake was not satisfactory (Mowat, 1971). Similarly, an alkali-treated straw silage having a pH range of 6.8 to 4.8 resulted in reduced intake (Lampila, 1963). Partial neutralization of 4% sodium hydroxide-treated corn stover and corn

cob silages by potassium and ammonium chloride has demonstrated improved animal response (Koers, Klopfenstein and Woods, 1969; Koers, Woods and Klopfenstein, 1970). Other reports have indicated acceptable animal performance from corn stover silages having 3, 4 and 5% sodium hydroxide without neutralization (Krause, Klopfenstein and Woods, 1968; Klopfenstein et al., 1972). These same reports showed improvement in digestion of alkali-treated stover silage but not whole corn plant, indicating more benefit for treating low quality roughages. A combination 3% sodium and 3% ammonium hydroxide added to a straw silage resulted in less ammonia losses than when ammonium hydroxide was used alone (Kormscikov, 1968).

Of the previously cited reports regarding chemical additives, one makes mention of organic acids formed in the silages (Goering et al., 1973). Straw silage having the chloride additive had a pH of 4.7 with greater lactic acid and less acetic, propionic and butyric acid than did the untreated control silage. The silage having the chlorite additive resulted in a pH of 3.7 and no detectable lactic or propionic acid and only a small amount of acetic and butyric acids. The authors stated this indicated a limited fermentation. In contrast, corn stover silage having increments of 2, 3 and 4% sodium hydroxide added on a dry matter basis (Buchanan-Smith et al., 1972) showed higher silage pH and increases in lactic and acetic acids while levels of propionic and butyric were reported

as minimal. These silages were fermented in small plastic bags for 50 days and the dry matter was unaffected.

Numerous enzyme and mold preparations have been used as additives in silage making (Owen, 1971) to promote lactic acid production. Ensiling wheat-ryegrass with 0.5% cellulase (McCullough, 1966) allowed increased acetic and decreased lactic acid in the silage while effects upon milk production were inconsistent. The use of various cellulase or Lactobacillus casei preparations as predigestors of cellulose in sugar cane bagasse silage resulted in limited degradation of the cell wall carbohydrate (Leatherwood et al., 1963). The high degree of lignin encasement and lack of buffering agents in the bagasse were listed as probable limiting factors. Fermentation stimulants containing the mold, Aspergillus oryzae, have been added to poplar bark silage without apparent effect (Enzmann, Goodrich and Meiske, 1969; Robertson, Beacom and Shiels, 1971). The effect of a lactobacterial product ensiled with corn stalks was obscured by other additives included (Calancea and Mosolova, 1963).

#### Mineral and Organic Acids

The addition of mineral acids (Virtanen, 1933) has been used to preserve silages of high moisture and low soluble carbohydrate content. In rice straw silage, described as a wet and green

crop residue following harvest (Paci, 1955), chemical composition was unchanged following a 98-day mineral acid preservation. Phosphoric acid has been used both as a preservative and source of phosphorus (Johnson et al., 1940; Bergner and Lange, 1970). Excesses of mineral acids have been shown to cause acidosis and marked increases in urinary calcium and nitrogen excretion in cattle (Crasemann, 1937; Lang and Bergner, 1970; L'Estrange and Murphy, 1972).

Considerable attention has been directed to organic acid additives, in particular formic acid. This acid has been used extensively in the preservation of grass silages (Breirem et al., 1959, Syrjala, 1972; Wilson and Wilkins, 1972). Increased fiber digestion in corn silage but lowered digestibility in sorghum-sudan silage has been related to addition of 0.5% formic acid (Fisher, Lessard and Lodge, 1971; Waldo, Keys and Gordon, 1973a). In vitro digestion of wheat straw silage was not significantly increased following 8 days treatment with formic acid (Goering et al., 1973). Stimulation of lactic acid formation and silage preservation appears as the major benefits from formic acid additives.

#### Nitrogen Content

The inherent nitrogen content in straw and other high-fiber crop residues is commonly low. Preformed proteins in silage are

subject to considerable degradation during ensilation with resultant formation of undesirable amines and higher fatty acids (Barnett, 1954; Wieringa, 1960). The possible use of formic-formaldehyde additives to protect against protein hydrolysis in the silo as well as in the rumen has recently been demonstrated (Waldo, Keys and Gordon, 1973b).

Non-protein nitrogen has been shown to be an adequate nitrogen source for silage microbes (Brady, 1966). Various non-protein nitrogen additives such as urea, ammonium polyphosphate and others have been used successfully to stimulate organic acid production in silage as well as being a nitrogen source for the ruminant (Huber, Thomas and Emery, 1968; Colenbrander, Muller and Cunningham, 1971; Shirley et al., 1972; Huber and Santana, 1972). A commercial ammonia-molasses-phosphoric acid additive has evolved and has given generally favorable results.

Relatively few studies have been conducted to compare nitrogen additives in silages. Ensilation of corn silage or corn stalklage with urea or biuret has been compared (Karr et al., 1965; Condon et al., 1969; Owens, Meiske and Goodrich, 1970). A similar pattern of silage characteristics were observed in these comparisons in that urea extended fermentation longer than biuret. As a consequence, generally greater lactic and total organic acid production, but higher pH and more nitrogen and dry matter losses were

associated with urea additives.

### Minerals and Vitamins

In addition to the minerals that have been included in previously mentioned cell wall-degrading solutions and the minerals acids, there are two other elements used extensively as silage additives. Calcium and sulfur have also been incorporated into silages in compounds having a primary objective of affecting fermentation rather than adding minerals per se.

The adding of calcium carbonate, often referred to as limestone, is associated with a buffering action to silage fermentation resulting in greater organic acid production but a higher pH (Nicholson and Cunningham, 1964; McCullough, Sisk and Smart, 1970). A lack of soluble carbohydrates can result in undesirably high silage pH in mature plant ensilages having limestone and/or urea additives (Essig, 1968; Johnson, DeFaria and McClure, 1971). Generally, animal performance has improved when fed silages having limestone. Ensiled calcium carbonate or calcium sulfate has been indicated as potentially useful mineral supplement sources (Condon et al., 1969; Clarisse, Corrick and Hobbs, 1972).

The principal form of additive sulfur used to date in silages has been the bacteriostat, sodium metabisulfite. This has appeared of benefit in reducing ammonia losses from low dry matter-high



protein silages; however, when used in appreciable amounts with high dry matter plant material, poor quality silage has resulted (Owen, 1971). Ammonium bisulfate was of some benefit for preservation of alkali-treated straw silage (Lampila, 1963).

Elemental sulfur and ammonium sulfate have been reported to stimulate lactic acid fermentation in corn cob or corn stalk silages and to increase the beneficial effect of ammonial additives (Calancea and Mosolova, 1961, 1963). In contrast, sodium metabisulfite has markedly increased gas losses from corn stalk silages having added urea, more so than with added biuret (Owens, Meiske and Goodrich, 1970).

Ascorbic acid and the vitamin A precursor, carotene, generally are degraded slowly during ensilation (Barnett, 1954). The content of these in crop residue silages is inherently low, with the possible exception of some corn stalk silages. Although some commercial preparations contain vitamins to "spike" silages, the actual benefit is not clear, and supplemental vitamin feeding appears more reliable.

## EXPERIMENTAL PROCEDURES

A series of experiments utilizing laboratory silos and in vitro digestion, followed by animal metabolism and performance trials, were used to evaluate the effects of various additives ensiled with annual ryegrass straw. Straw used in all trials was chopped to have an approximate average length of 5 centimeters.

### Laboratory Silo Trials

Chopped straw from a common stockpile was used in all laboratory trials and had the following chemical composition: acid detergent fiber, 44.9%; acid detergent lignin, 7.3%; residual ash, 3.1%; cell wall constituents, 74.3% and crude protein, 3.8%.

Wide mouth .95 l glass jars with self-sealing lids served as laboratory silos. Straw, 125 g to each silo, and other materials were packed in the jars with the aid of a large porcelain pestle, which was rinsed off into the jar with the respective treatment solution. The mini-silos were kept in a dark closet at room temperature (25<sup>o</sup> C).

Trial I consisted of a 3 x 3 factorial arrangement of treatments in which straw was ensiled with either water, 1% hydroxide solution or water-enzyme solution. The hydroxide solution contained 0.5 g sodium hydroxide (NaOH) and 0.5 g potassium hydroxide (KOH)/

100 ml water. The water-enzyme solution contained cellulase, having 4000 units activity/g, and was added at a 0.5% (w/w) rate to straw dry matter. Water or solutions were added at 450 ml/100 g straw to approximate a 22% dry matter in silages. Thus, 4.5% hydroxide was added w/w to straw. The three solutions contained either 0, 10 or 20% cane molasses w/w to straw. The molasses was a standardized commercial product having 79.5° Brix and 70% TDN values. Two mini-silos/treatment were used in both trials I and II and were ensiled for 20 days (table 1). In these and subsequent trials, a percent chemical or other additive represents actual grams added per 100 g of straw dry matter.

A 2 x 3 x 2 factorial design was used in trial II as straw was ensiled with either water and 20% molasses or 4.5% hydroxide in solution and 20% molasses. Each solution contained either no non-protein nitrogen (NPN), or 1% (w/w) urea having 281% protein equivalent, or 1.2% (w/w) biuret having 230% protein equivalent. Elemental sulfur was either withheld or added at a rate of .1% (w/w) to straw dry matter in the respective treatments.

Trial III involved a 2 x 3 x 2 factorial arrangement in which straw was ensiled with either water and 20% molasses plus 1% urea, or 4.5% hydroxide in solution with 20% molasses plus 1% urea. Each solution contained either 0.5% concentrated formic acid, or 0.5% limestone or no additional additive. These agents were added

Table 1. Straw and Additives Ensiled in Trials I and II

Ingredients				Treatments						
				Trial I <sup>a</sup>						
Additives (3)	450 ml water/100 g <u>straw</u>			450 ml 1% hydroxide <u>solution/100 g straw</u>			450 ml water with 0.5% <u>enzyme<sup>b</sup>/100 g straw</u>			
% molasses (3)	0	10	20	0	10	20	0	10	20	
				Trial II <sup>a</sup>						
Additives (2)	water + 20% molasses					4.5% hydroxide in solution + 20% molasses				
NPN source (3)	<u>none</u>	<u>urea</u>	<u>biuret<sup>c</sup></u>				<u>none</u>	<u>urea</u>	<u>biuret<sup>c</sup></u>	
.1% sulfur <sup>d</sup> (2)	- +	- +	- +				- +	- +	- +	

<sup>a</sup> Two mini-silos/treatment<sup>b</sup> "Cellulase 4000" courtesy Miles Laboratory, Elkhart, Indiana<sup>c</sup> "Kedlor" biuret courtesy Dow Chemical Co., Midland, Michigan<sup>d</sup> Elemental sulfur

Table 2. Straw and Additives Ensiled in Trials III and IV

Ingredients		Treatments												
Trial III <sup>a</sup>														
Solutions (2)	Water + 20% molasses + 1% urea						4.5% hydroxide in solution + 20% molasses + 1% urea							
Additives (3)	<u>none</u>	<u>formic acid<sup>b</sup></u>		<u>limestone</u>		<u>none</u>	<u>formic acid</u>		<u>limestone</u>					
Days ensiled (2)	20	40	20	40	20	40	20	40	20	40	20	40		
Trial IV <sup>c</sup>														
Additives (2)	Water + 20% molasses + 1% urea						4.5% hydroxide in solution + 20% molasses + 1% urea + 0.5% limestone							
Days ensiled (7)	0	2	4	8	16	32	64	0	2	4	8	16	32	64

<sup>a</sup> Two mini-silos/treatment<sup>b</sup> Concentrated reagent grade formic acid<sup>c</sup> Three mini-silos/treatment

on a w/w basis with straw. Each treatment was ensiled for both 20 and 40 days with 2 mini-silos/treatment and days ensiled (table 2).

A 2 x 7 factorial design was used in trial IV as straw was ensiled with either water + 20% molasses + 1% urea + 0.5% limestone or 4.5% hydroxide in solution + 20% molasses + 1% urea + 0.5% limestone. Three mini-silos/treatment were prepared for 0, 2, 4, 8, 16, 32 and 64 days ensilation (table 2).

Upon completion of each trial the mini-silos were opened singly in the same order that they were sealed. Immediately after opening, effluent was poured or pressed out by porcelain pestle and pH was measured with a potentiometer. The silage was reconstituted with the fluid, then random subsamples were taken throughout the jar with rubber tongs. The samples were freeze-dried for 24 hr to determine percent dry matter, then stored for chemical analyses and in vitro digestion.

#### Animal Metabolism Trial

Eight crossbred wethers averaging 35.5 kg were used in a 2 x 2 Latin square designed metabolism trial. Animals were stratified by weight and randomly assigned to the two treatments in period I, then switched to the other treatment in period II. The two treatments were straw ensiled with water + 20% molasses

+ 1% urea + 0.5% limestone, and straw ensiled with 4.5% hydroxide in solution + 20% molasses + 1% urea + 0.5% limestone (table 3).

Materials used were in the same proportions as the laboratory trials and were ensiled in 122 l plastic barrels. Several barrel-silos were prepared for each treatment. Lids equipped with bunsen valves, were sealed with petroleum jelly and weights approximating 15 kg were placed on top each lid.

The barrel-silos were closed for a minimum 45 days then opened singly as needed for each of the two periods of the trial. As each silo was opened, the entire silage mass was inverted so that the top portion of straw silage was submerged in the effluent at the bottom of the barrel and the bottom layer of silage was fed first. The animals were fed at constant intake to approximate maintenance level. Four portions of silage were offered daily after being strained in a large plastic colander to remove excess free fluid. A commercial trace mineralized salt (table 3) and water were offered free-choice. Intakes and refusals were recorded daily. Periodic samples of silage were sealed in plastic bags and transferred to the laboratory to be freeze-dried for dry matter determination and chemical analyses (table 4). Silage juices were periodically measured with a pH potentiometer.

The trial was conducted in metabolism cages with 14-day preliminary and 7-day total collection periods. Daily fecal output

Table 3. Straw Silages Used in Lamb Metabolism Trial

Wether lambs		Periods	
	I		II
4	Untreated <sup>a</sup>		Treated <sup>b</sup>
4	Treated		Untreated

<sup>a</sup> Untreated straw silage with water, 20% molasses, 1% urea and 0.5% limestone

<sup>b</sup> Treated straw silage with 4.5% hydroxide in solution, 20% molasses, 1% urea, and 0.5% limestone

Trace mineralized salt: 98.5% NaCl, .25% Zn, .20% Mn, .125% Fe, .025% Cu, .005% I, .005% Co.

Table 4. Chemical Composition of Straw Silages Fed in Lamb Metabolism Trial<sup>a</sup>

Item	Silage type	
	Untreated <sup>b</sup>	Treated <sup>b</sup>
pH	3.9	5.1
Dry matter	27.8	28.4
<u>% of dry matter</u>		
Crude protein	7.6	7.7
Acid detergent fiber	41.0	39.9
Acid detergent lignin	6.5	6.8
Residual ash	2.5	1.8
Cell wall constituents	63.0	59.6
Lactic acid	2.7	3.1
Acetic acid	5.6	7.4
Propionic acid	---	1.6
Butyric acid	0.8	12.9

<sup>a</sup> Average value of four analyses/treatment of composite samples

<sup>b</sup> Described in table 3

of each animal was weighed and 10% weight aliquots were frozen in plastic bags, then thawed at a later date for dry matter and chemical analyses. Daily urine excretions of each animal were collected in plastic buckets containing 10 ml concentrated  $\text{H}_2\text{SO}_4$ . Urine volume and specific gravity, measured with a hydrometer, were recorded and 10% volume aliquots refrigerated in glass jars for subsequent nitrogen analysis.

Following completion of each collection period, rumen fluid was aspirated by stomach tube 4 hours after fresh feed was offered. Fluid was collected into 50 ml glass jars containing 2 ml of 2N  $\text{H}_2\text{SO}_4$ . Fluid was then centrifuged at 3000 rpm for 10 minutes and supernatant frozen for volatile fatty acid (VFA) analyses.

#### Animal Performance Trial

A 3 x 2 factorial design was used to measure body weight gains from feeding 3 types of silage with 2 types of supplement (table 5). Direct cut ryegrass was chopped and ensiled in a concrete bunker silo. Chopped ryegrass straw was ensiled with either water, 20% molasses, 1% urea and 0.5% limestone, or with 4.5% NaOH, 20% molasses, 1% urea and 0.5% limestone. The straw silages were prepared in trench silos lined with plastic sheet roll. Additives were layered on the straw intermittently and soaked with water as chopped straw was blown into the silo. Water was added to approximate a



30% dry matter in the straw silages. Silages were allowed to ferment a minimum of 60 days before commencing the feeding trial. Weekly silage samples of that being fed were frozen in sealed plastic bags until chemically analyzed (table 6).

Thirty Hereford heifers, averaging 234 kg, were stratified by weight and randomly allotted to the six treatments in penlots of five. The animals received ryegrass hay during a 10 day preliminary period. All animals were offered trace mineralized salt (table 3) and water free-choice throughout the trial. Either a semi-solid molasses-urea supplement containing 20% vegetable fat or a dry supplement of ground barley and cottonseed meal was offered at a rate of .9 kg/animal/day with each of the silages. All animals received an additional fattening supplement during the final 30 days of the trial at the rate of .9 kg/animal/day (tables 5 and 6).

#### Chemical Analyses and In Vitro Procedure

Dried feed and feces samples were ground in a Wiley mill to pass a 60 mesh screen then analyzed for acid detergent fiber (ADF), acid detergent lignin (ADL), residual ash and cell wall constituents (CWC) by the micro-method of Waldren (1971). Crude protein was determined in feed and feces by micro-Kjeldahl and in urine by macro-Kjeldahl procedures of the A. O. A. C. (1965).

The freeze-dried and ground silage samples from trials I-IV

Table 5. Silages and Supplements Fed in Heifer Performance Trial.

Item	Treatments <sup>a</sup>					
	Ryegrass		Untreated straw <sup>b</sup>		Treated straw <sup>b</sup>	
Silage type (3)						
Supplement (2)	semi-solid <sup>c</sup>	dry <sup>d</sup>	semi-solid	dry	semi-solid	dry

<sup>a</sup> All animals, free choice trace mineralized salt (table 3); final 38 days additional supplement: 30% steam rolled wheat, 40% steam rolled barley, 10% beet pulp, 5% mustard, 5% molasses, .5% urea and .5% salt

<sup>b</sup> Described in table 3

<sup>c</sup> Semi-solid supplement: 20% vegetable fat+ emulsifier and starch, 7% urea, remainder molasses

<sup>d</sup> Dry supplement: 65% ground barley and 35% cottonseed meal

Table 6. Chemical Composition of Silages and Supplements Fed in Heifer Performance Trial.

Item	Silage types		
	Ryegrass	Untreated straw <sup>b</sup>	Treated straw <sup>b</sup>
pH	4.2	4.0	5.2
Dry matter	36.8	39.9	37.4
<u>% of dry matter<sup>a</sup></u>			
Crude protein	12.0	7.9	7.0
Acid detergent fiber	39.2	48.1	49.5
Acid detergent lignin	7.6	8.9	9.2
Residual ash	2.5	2.5	2.4
Cell wall constituents	52.3	69.0	72.6
Lactic acid	3.0	1.2	1.0
Acetic acid	8.2	2.4	6.1
Propionic acid	---	---	0.8
Butyric acid	---	1.6	14.2
<u>Supplements</u>			
	<u>Semi-solid</u>	<u>Dry</u>	<u>Fattening</u>
Crude protein, %	20.0	20.0	10.2
Vit. A, IU/kg	9,090	1,136	32,500
Vit. D, IU/kg	6,818	273	---
Vit. E, IU/kg	9	23	---

<sup>a</sup> Analyses from extracts of composited fresh frozen silage samples

<sup>b</sup> Described table 3

were fermented in triplicate for 24 hr to determine in vitro dry matter digestion (IVDMD). Inoculum was obtained from a Holstein steer maintained on an ad libitum ryegrass hay diet. Solids and liquid were taken by hand from the ventral and caudoventral sacs, strained through four layers of surgical gauze, and liquid was collected in a pre-warmed thermos. The procedure for fermentation and IVDMD was essentially that outlined by Kutches, Church and Duryee (1970).

Following incubation, trial IV samples were acidified then refrigerated overnight to promote settling of solids. Five ml of liquid, visibly free of solids, were then aspirated and frozen for subsequent VFA analyses while the remaining contents of each vessel were used for IVDMD.

Aqueous extracts of fresh silage samples from trial IV and the animal digestion trial and of fresh frozen composited samples of silage from performance trial were prepared according to the method of Lopez et al. (1970). Extracts were analyzed for total lactate by the procedure of Barnett (1951). The extracts, as well as in vitro rumen fluids from the animal trial were analyzed for VFA by the method of Erwin et al. (1961). Conditions for VFA analyses were basically those described by Kutches, Church and Duryee (1970) and quantitated by the procedure of Baumgardt (1964).

### Statistical Analyses

Data were subjected to analysis of variance for factorial and Latin square designs and means compared by the least significant differences (LSD) procedures outlined by Steel and Torrie (1960).

## RESULTS AND DISCUSSION

### Laboratory Evaluation of Additives in Straw Silage

#### Trial I

Some characteristics of ryegrass straw silage as affected by ensiling with either water, hydroxide, enzyme and/or molasses are presented in table 7. Following 20 days ensilation, the mini-silo contents prepared with water or a 1% hydroxide solution (4.5% w/w hydroxide to straw) did not have a desirable fermentation as evidenced by the higher ( $P < .01$ ) pH values of 5.5 and 6.7, respectively. The inclusion of .5 g cellulase/100 g straw lowered ( $P < .01$ ) the pH to 4.8. The addition of 10% molasses further decreased ( $P < .01$ ) pH values while 20% molasses gave a concomitant pH reduction only with the straw and water ensilage ( $P < .01$ ).

Straw silage dry matter or residual ash were not significantly altered by the additives used in these mini-silos. The ensiling of straw with water or hydroxide solution or water-enzyme did not alter ADF, ADL or CWC values; however, the percentage of these were lowered ( $P < .01$ ) when 10% molasses was incorporated with the hydroxide or water-enzyme solutions. This fermentative effect was not attained in straw ensiled with water until 20% molasses was included ( $P < .01$ ). A further reduction ( $P < .01$ ) in silage CWC, but

Table 7. Some Characteristics of Ryegrass Straw Silage as Affected by Additions of Water, Hydroxide, Enzyme and Molasses.<sup>a</sup>

Characteristics	Additives % molasses	Water			4.5% hydroxide in solution <sup>b</sup>			water-enzyme <sup>c</sup>		
		0	10	20	0	10	20	0	10	20
Silage pH		5.5 <sup>i</sup>	4.0 <sup>f</sup>	3.6 <sup>e</sup>	6.7 <sup>j</sup>	4.4 <sup>g</sup>	4.2 <sup>fg</sup>	4.8 <sup>fg</sup>	4.4 <sup>g</sup>	4.3 <sup>fg</sup>
Silage dry matter, %		23.8	23.5	23.9	25.1	24.0	24.4	24.6	23.5	23.8
<u>% of dry matter</u>										
Acid detergent fiber		47.7 <sup>g</sup>	48.6 <sup>g</sup>	43.9 <sup>e</sup>	48.9 <sup>g</sup>	44.1 <sup>e</sup>	42.7 <sup>e</sup>	47.7 <sup>g</sup>	46.2 <sup>f</sup>	44.3 <sup>e</sup>
Acid detergent lignin		8.9 <sup>fg</sup>	9.6 <sup>g</sup>	7.4 <sup>e</sup>	9.0 <sup>fg</sup>	8.3 <sup>f</sup>	6.9 <sup>e</sup>	9.3 <sup>g</sup>	6.9 <sup>e</sup>	6.6 <sup>e</sup>
Residual ash		3.1	2.8	2.7	2.7	2.8	2.6	2.5	3.1	2.4
Cell wall constituents		74.9 <sup>h</sup>	74.7 <sup>h</sup>	72.3 <sup>g</sup>	74.1 <sup>h</sup>	68.6 <sup>f</sup>	64.6 <sup>e</sup>	75.1 <sup>h</sup>	72.1 <sup>g</sup>	69.9 <sup>fg</sup>
Crude protein		3.7 <sup>e</sup>	4.3 <sup>f</sup>	4.8 <sup>g</sup>	4.3 <sup>f</sup>	4.5 <sup>fg</sup>	4.8 <sup>g</sup>	4.3 <sup>f</sup>	4.5 <sup>fg</sup>	4.8 <sup>g</sup>
IVDMD (24 hr), %		36.2 <sup>e</sup>	39.8 <sup>f</sup>	39.8 <sup>f</sup>	45.4 <sup>h</sup>	51.5 <sup>i</sup>	54.9 <sup>j</sup>	42.1 <sup>g</sup>	42.4 <sup>g</sup>	44.6 <sup>h</sup>

<sup>a</sup> Means based on two mini-silos/treatment with two samples/silo

<sup>b</sup> Solution described on p. 14

<sup>c</sup> "Cellulase 4000" courtesy Miles Laboratory, Elkhart, Indiana

<sup>d</sup> Native straw had 32.8% IVDMD

e, f, g, h, i, j Means in same row with unlike superscripts differ significantly ( $P < .01$ )

not ADF or ADL, was observed when 20% molasses was added to the hydroxide solution, suggesting increased hemicellulose degradation. The crude protein content of straw ensiled with water was lower ( $P < .01$ ) than other treatments. Increases in silage protein values corresponded to molasses additions and the highest ( $P < .01$ ) values were the same for ensiled material with water, hydroxide or water-enzyme solutions having 20% molasses.

The 24 hr IVDMD of hydroxide-treated straw silage was greater ( $P < .01$ ) than other treatments, while enzyme-treated silage presented a higher ( $P < .01$ ) value than straw ensiled only with water. Adding 10% molasses at ensiling with straw and water or straw and hydroxide solution gave increases ( $P < .01$ ) of 3.6 and 6.1 percentage units, respectively. The addition of 20% molasses to straw and hydroxide resulted in 3.4 more ( $P < .01$ ) units of IVDMD. An increase ( $P < .01$ ) of 4.2 and 4.5 percentage units were derived from ensiling 20% molasses with straw and enzyme as compared to this treatment having 10% or no molasses, respectively.

The ensiling of corn stover or barley straw with 6% hydroxide w/w has presented pH values of 7 and 7.5, respectively (Mowat, 1971). When these silages were fed as 50% of the ration, intake and body gains were reduced. Under controlled mini-silo conditions, corn stover silage dry matter percent has shown no change when ensiled with 4% hydroxide and resulted in 5.25 pH compared to a

4.65 control pH (Buchanan-Smith et al., 1972).

Improved IVDMD has been demonstrated in crop residues ensiled with hydroxide (Krause, Klopfenstein and Woods, 1968; Klopfenstein et al., 1972; Koers, Prokop and Klopfenstein, 1972) and no intake problems have been reported when fed with protein supplements. Ensiling straw with chlorine, chloride or chlorites has increased IVDMD (Yu Yu, Thomas and Emery, 1970) while in vivo results were not favorable (Yu Yu, Thomas and Emery, 1971). Although IVDMD was improved by ensiling straw with chlorite (Goering et al., 1973), no lactic acid was detected in the silage and the authors stated that the use of this chemical was limited by mixing and safety problems.

Ensiling alfalfa or sugar cane bagasse with up to .5% cellulase preparations has improved in vitro cellulose utilization while not altering silage pH significantly (Leatherwood et al., 1963). Ensilation of .5% cellulase with wheat-ryegrass did not appreciably affect milk production (McCullough, 1966). The inclusion of a fermentation stimulant containing Aspergillus oryzae with poplar bark or low quality sorghum forage at ensiling time promoted fermentation but did not improve bark utilization (Enzmann, Goodrich and Meiske, 1969; Robertson, Beacom and Shiels, 1971; Sherrod and Hollingsworth, 1971).

Limited information regarding molasses additions to



coarse-textured roughages at ensilation indicates that 10-15% improves silage quality and preservation (Barker and Levitt, 1969; O'Donovan, Chen and Lee, 1972). Ensiling rice straw with 5% molasses gave a 3.9 silage pH, while added urea raised the pH to 5.2 as compared to a 4.9 pH in control straw silage (Sutoh and Uchida, 1970). These authors scored the silage aspects good, fair and poor for the respective treatments. The aforementioned observations and that of the current trial indicate that the added molasses may not promote utilization of the straw per se but would be of value by enhancing and preserving desirable silage characteristics, particularly if other additives such as non-protein-nitrogen are to be incorporated.

## Trial II

Some effects of ensiling non-protein-nitrogen and/or sulfur with ryegrass straw are presented in table 8. The addition of .1% sulfur w/w to straw ensiled with water + 20% molasses raised ( $P < .01$ ) the pH from 4.4 to 4.8, while in material ensiled with hydroxide solution + 20% molasses the pH was elevated ( $P < .01$ ) from 4.8 to 5.2. The inclusion of 1% urea w/w to straw with water + molasses at ensiling resulted in a pH rise ( $P < .01$ ) to 4.9. Combining sulfur with NPN sources altered the silage pH only when included with hydroxide solution + molasses and urea. The

Table 8. Effects of Non-protein Nitrogen and Sulfur Upon Some Ryegrass Straw Silage Characteristics<sup>a</sup>

Characteristics	NPN source <sup>d</sup> .1% sulfur	Water + 20% molasses						4.5% hydroxide in solution <sup>b</sup> + 20% molasses					
		none		1% urea		1.2% biuret <sup>c</sup>		none		1% urea		1.2% biuret <sup>c</sup>	
		-	+	-	+	-	+	-	+	-	+	-	+
Silage pH		4.4 <sup>f</sup>	4.8 <sup>g</sup>	4.9 <sup>gh</sup>	4.6 <sup>fg</sup>	4.3 <sup>f</sup>	4.4 <sup>f</sup>	4.8 <sup>g</sup>	5.2 <sup>h</sup>	4.8 <sup>g</sup>	5.9 <sup>i</sup>	5.0 <sup>gh</sup>	5.0 <sup>gh</sup>
Silage dry matter, %		21.9	23.0	22.2	22.4	21.7	21.6	22.0	21.8	23.4	22.1	22.1	22.0
<u>% of dry matter</u>													
Acid detergent fiber		41.7 <sup>h</sup>	40.6 <sup>h</sup>	41.6 <sup>h</sup>	41.1 <sup>h</sup>	41.6 <sup>h</sup>	40.8 <sup>h</sup>	39.3 <sup>g</sup>	39.3 <sup>g</sup>	39.6 <sup>gh</sup>	38.7 <sup>g</sup>	37.4 <sup>f</sup>	37.7 <sup>f</sup>
Acid detergent lignin		6.7	6.6	7.1	7.3	6.6	7.0	7.0	6.5	6.8	7.0	6.6	6.8
Residual ash		3.0	3.1	2.9	2.8	2.7	2.8	2.6	2.8	2.8	3.0	2.9	2.6
Cell wall constituents		67.4 <sup>i</sup>	65.7 <sup>hi</sup>	66.9 <sup>i</sup>	66.1 <sup>hi</sup>	66.4 <sup>hi</sup>	64.9 <sup>h</sup>	60.2 <sup>g</sup>	60.0 <sup>g</sup>	59.0 <sup>fg</sup>	57.5 <sup>f</sup>	57.2 <sup>f</sup>	57.2 <sup>f</sup>
Crude protein		4.1 <sup>f</sup>	4.5 <sup>f</sup>	6.4 <sup>g</sup>	6.1 <sup>g</sup>	7.2 <sup>h</sup>	7.4 <sup>h</sup>	4.2 <sup>f</sup>	4.6 <sup>g</sup>	6.1 <sup>g</sup>	7.4 <sup>h</sup>	7.4 <sup>h</sup>	7.2 <sup>h</sup>
IVDMD (24 hr), %		43.1 <sup>j</sup>	43.1 <sup>j</sup>	45.2 <sup>k</sup>	44.1 <sup>jk</sup>	43.9 <sup>jk</sup>	42.7 <sup>j</sup>	54.2 <sup>m</sup>	50.1 <sup>l</sup>	55.8 <sup>n</sup>	53.0 <sup>m</sup>	49.4 <sup>l</sup>	50.4 <sup>l</sup>

<sup>a</sup> Means based on two mini-silos/treatment with two samples/silo

<sup>b</sup> Solution described on p. 14

<sup>c</sup> "Kedlor" biuret courtesy Dow Chemical Corp., Midland, Michigan

<sup>d</sup> Elemental sulfur

<sup>e</sup> Native straw had 33.4% IVDMD

<sup>f, g, h, i</sup> Means in same row with unlike superscripts differ significantly ( $P < .01$ )

<sup>j, k, l, m, n</sup> Means in same row with unlike superscripts differ significantly ( $P < .05$ )

resultant pH change was from 4.8 to 5.9 ( $P < .01$ ). As the mini-silos were opened following 20 days fermentation, the jars containing added sulfur had a noticeable sulfur odor. Some top spoilage and minor layered spoilage within contents was evident in the hydroxide treated silages having sulfur.

Silage dry matter, residual ash or ADL were not appreciably altered by treatments. The ADF and CWC values were lowered ( $P < .01$ ) when 1.2% biuret w/w was added in combination with sulfur to straw and water + molasses. This effect was also evident ( $P < .01$ ) in the straw and hydroxide + molasses silage when biuret was added alone or in combination with sulfur. A reduction ( $P < .01$ ) in CWC, but not ADF, resulted from ensiling urea and sulfur with the hydroxide + molasses straw silage. These observations suggest a possible interaction of these additives with the straw hemicellulose fraction.

Crude protein content of silages was increased ( $P < .01$ ) by adding NPN at ensiling time. This effect was more pronounced ( $P < .01$ ) with biuret than with urea, indicating a loss of ammonia from urea when the mini-silos were opened. This loss was not as apparent when urea was combined with sulfur in the straw and hydroxide + molasses silage.

An improvement ( $P < .05$ ) in 24 hr IVDMD of either straw and water + molasses silage or straw and hydroxide + molasses silage

resulted when urea was added during ensilation. The addition of biuret did not improve IVDMD and a reduction ( $P < .05$ ) was observed following its incorporation with straw and hydroxide + molasses. The effect of added sulfur in these silages revealed a tendency toward reduction of IVDMD with a depression being significant ( $P < .05$ ) in the straw and hydroxide + molasses silages with or without urea.

The use of NPN in silages has generally given satisfactory results, due in part to increased dietary nitrogen and more organic acids produced from extended fermentation (Huber, Thomas and Emery, 1968; Owen, 1971; Shirley et al., 1972; Huber and Santana, 1972). Comparing the ensilation of urea to biuret with corn silage has shown greater lactic and total organic acid production, more dry matter and nitrogen losses and generally higher pH values from urea-treated silages (Karr et al., 1965; Condon, et al., 1969). Similar results were observed when comparing urea and biuret ensiled with corn stalkage (Owens, Meiske and Goodrich, 1970). Increasing the amount of urea to .75% at ensiling with corn stover improved IVDMD and lowered structural composition values of the silage (Colenbrander et al., 1971; Colenbrander, Muller and Cunningham, 1971).

It has been demonstrated that sulfur supplementation will improve fiber digestion of corn fodder containing urea (Barton, Bull

and Hemken, 1971; Bull and Vandersall, 1973; Grieve, Merrill and Coppock, 1973). The major sulfur additive used in silages has been the bacteriostat, sodium metabisulfite. It has been reported to reduce ammonia losses during silage fermentation; however, when used in silage material having more than 21% dry matter or at a 1% w/w level, poor quality silage has resulted (Owen, 1971).

Sodium hydroxide-treated straw, rinsed to have a 1% alkali residue in 20% dry matter material, has been ensiled with 0 to .8% w/w ammonium sulfate preservative solution to give a fermented product with a final pH range of 6.8 to 4.8 (Lampila, 1963).

Ammonium chloride, ammonium sulfate and elemental sulfur have been reported to stimulate lactic fermentation in corn stalks having molasses and a lacto bacterial product (Calancea and Mosolova, 1963). Delayed fermentation and increased gas production has been observed when sodium metabisulfite was ensiled with urea or biuret into corn stalklage. The effect was more prevalent with urea than biuret (Owens, Meiske and Goodrich, 1970). Additional reports indicate increased organic acid production when calcium sulfate and urea were added to corn silage in mini-silos (Condon et al., 1969) while sodium sulfate with urea-treated corn silage did not alter daily body weight gains (Chamberlain, Thomas and Felts, 1972).

### Trial III

Influences of formic acid or limestone upon ryegrass straw silage following 20 and 40 days ensilation are presented in table 9. The pH of straw silage having water + 20% molasses and 1% urea was lower ( $P < .01$ ) after 40 days fermentation than at 20 days. The time effect upon pH was not significant between 20 and 40 days fermentation of the straw silage having 4.5% hydroxide + 20% molasses and 1% urea. Ensiling .5% formic acid w/w with either type of silage for 20 days presented the lowest ( $P < .01$ ) pH values in the trial. The same pH level remained following 40 days fermentation of the silage having water + molasses and urea. An increased ( $P < .01$ ) pH did occur in the hydroxide + molasses and urea-treated silage at 40 days having formic acid, but this value was considerably lower ( $P < .01$ ) than without this acid.

The addition of .5% limestone w/w to the straw silage having water + molasses and urea resulted in a pH drop ( $P < .01$ ) from 4.8 at 20 days to 4.1 at 40 days. The hydroxide + molasses and urea-treated silage ensiled with limestone gave an increase ( $P < .01$ ) from 4.2 at 20 days to 4.5 at 40 days. These values were lower ( $P < .01$ ) than the same type silage without limestone at corresponding days of ensilation.

Silage dry matter was not appreciably affected by the amounts

Table 9. Effects of Formic Acid and Limestone on Ryegrass Straw Silage Ensiled 20 and 40 days.<sup>a</sup>

Characteristics	Additives Days ensiled	Water + 20% molasses + 1% urea						4.5% hydroxide in solution <sup>b</sup> + 20% molasses + 1% urea					
		None		.5% formic acid <sup>c</sup>		.5% limestone		none		.5% formic acid		.5% limestone	
		20	40	20	40	20	40	20	40	20	40	20	40
Silage pH		4.8 <sup>g</sup>	4.5 <sup>f</sup>	4.0 <sup>e</sup>	4.1 <sup>e</sup>	4.8 <sup>g</sup>	4.1 <sup>e</sup>	4.8 <sup>g</sup>	5.0 <sup>g</sup>	4.1 <sup>e</sup>	4.3 <sup>ef</sup>	4.2 <sup>ef</sup>	4.5 <sup>f</sup>
Silage dry matter, %		22.1	22.3	21.9	21.4	22.3	23.2	22.3	22.7	22.4	23.2	22.7	22.9
<u>% of dry matter</u>													
Acid detergent fiber		40.7 <sup>f</sup>	39.9 <sup>ef</sup>	42.3 <sup>g</sup>	42.5 <sup>g</sup>	40.0 <sup>ef</sup>	38.8 <sup>e</sup>	39.9 <sup>ef</sup>	41.9 <sup>fg</sup>	39.5 <sup>ef</sup>	40.3 <sup>ef</sup>	39.4 <sup>ef</sup>	41.3 <sup>fg</sup>
Acid detergent lignin		7.0 <sup>ef</sup>	7.0 <sup>ef</sup>	7.6 <sup>f</sup>	7.8 <sup>f</sup>	6.8 <sup>e</sup>	6.7 <sup>e</sup>	7.1 <sup>ef</sup>	7.5 <sup>ef</sup>	7.0 <sup>ef</sup>	7.2 <sup>ef</sup>	6.8 <sup>e</sup>	7.3 <sup>ef</sup>
Residual ash		3.1 <sup>f</sup>	1.3 <sup>e</sup>	3.0 <sup>f</sup>	1.3 <sup>e</sup>	2.8 <sup>f</sup>	1.3 <sup>e</sup>	3.2 <sup>f</sup>	1.5 <sup>e</sup>	2.8 <sup>f</sup>	1.5 <sup>e</sup>	2.4 <sup>ef</sup>	1.5 <sup>e</sup>
Cell wall constituents		65.9 <sup>ef</sup>	63.9 <sup>ef</sup>	67.4 <sup>f</sup>	67.2 <sup>f</sup>	64.6 <sup>ef</sup>	64.0 <sup>ef</sup>	61.0 <sup>e</sup>	63.8 <sup>ef</sup>	61.8 <sup>e</sup>	61.4 <sup>e</sup>	61.4 <sup>e</sup>	62.2 <sup>e</sup>
Crude protein		6.0 <sup>f</sup>	5.4 <sup>e</sup>	6.3 <sup>f</sup>	6.1 <sup>f</sup>	5.9 <sup>f</sup>	5.8 <sup>ef</sup>	6.3 <sup>f</sup>	6.3 <sup>f</sup>	6.0 <sup>f</sup>	6.3 <sup>f</sup>	5.8 <sup>ef</sup>	6.2 <sup>f</sup>
IVDMD (24 hr), %		42.4 <sup>e</sup>	42.4 <sup>e</sup>	39.7 <sup>e</sup>	40.0 <sup>e</sup>	42.4 <sup>e</sup>	46.4 <sup>f</sup>	54.6 <sup>g</sup>	52.4 <sup>g</sup>	52.3 <sup>g</sup>	51.5 <sup>g</sup>	54.3 <sup>g</sup>	53.2 <sup>g</sup>

<sup>a</sup> Means based on two mini-silos/treatment with two samples/silo.

<sup>b</sup> Solution described on p. 14

<sup>c</sup> Reagent grade

<sup>d</sup> Native straw had 33.1% IVDMD

<sup>e, f, g</sup> Means in same row with unlike superscripts differ significantly ( $P < .01$ )

of formic acid or limestone used in these mini-silos. Residual ash values were lower ( $P < .01$ ) following 40 days fermentation than at 20 days. This was observed in either type of silage, with or without formic acid or limestone. One exception was the hydroxide-treated silage with limestone.

Ensiling formic acid for 20 or 40 days with straw having water + molasses and urea gave higher ( $P < .01$ ) values for ADF, ADL and CWC as compared to this type silage with or without limestone. The addition of formic acid or limestone to the hydroxide + molasses and urea-treated silage did not alter ADF, ADL or CWC values and respective differences between 20 and 40 days fermentation were not significant. Silage crude protein content was not appreciably changed by treatments or ensilation time, except a lower ( $P < .01$ ) value in 40-day silage having water + molasses and urea.

Increased ( $P < .01$ ) 24 hr IVDMD was observed in silage having water + molasses and urea when ensiled for 40 days with limestone as compared to the same treatment with 20 days ensilation or the same type silage with or without formic acid. The addition of limestone or formic acid to hydroxide + molasses and urea-treated silage did not present a significant effect on IVDMD following 20 or 40 days ensilation.

It is of interest to note the lower residual ash values in silages of the present trial after 40 days ensilation as compared to material



ensiled for 20 days. The differences may reflect a removal of plant material silica compounds following extended fermentation. A relationship with structural carbohydrates has been suggested in earlier reports with non-ensiled material (Archibald, 1924; Van Soest and Lovelace, 1969).

Silages having urea and some form of soluble carbohydrate, such as molasses, can have a prolonged fermentation with eventual lowering in pH (Barnett, 1954; Schaadt and Johnson, 1968; Essig, 1968). Ensiled material with excess buffering capacity and/or lack of soluble carbohydrate can result in an elevated final pH (Lampila, 1963; Sutoh and Uchida, 1969; Johnson, DeFaria and McClure, 1971).

Formic acid has proven useful in controlling fermentation to a preservative acidic level (Breirem et al., 1959; McCullough, 1972; Wilson and Wilkins, 1972; Syrjala, 1972). Improved cell wall digestion has been reported in some formic acid-treated silages, while a reduction has been observed in material with relatively more cell wall constituents (Fisher, Lessard and Lodge, 1971; McCullough, 1972; Waldo, Keys and Gordon, 1973). A stabilization of the plant cell wall structure was suggested to occur with rapid acidification of the silage. IVDMD of wheat straw was not improved by addition of formic acid with 8 days storage (Goering et al., 1973). The composition of rice straw was reported to be essentially unchanged following 98-day ensilation with mineral acids, and the final silage pH

was 3.8 (Paci, 1955). These observations lend support to the lower IVDMD of straw silage with added formic acid in the present trial.

The addition of limestone to silages is generally associated with a higher pH and organic acid production relative to ensilage without this additive (Dexter, 1966; Schaadt and Johnson, 1968; Essig, 1968; McCullough, Sisk and Smart, 1970; Johnson, DeFaria and McClure, 1971). Dry matter digestion has been reported unaffected by limestone, with or without urea (Nicholson and Cunningham, 1964; Essig, 1968). Others have observed improved cellulose digestion and animal performance with the added urea-limestone combination and indicated ensiled limestone as a useful mineral supplement source (Condon et al., 1969; Johnson, DeFaria and McClure, 1971; Clarisse, Corrick and Hobbs, 1972). The inclusion of limestone to ryegrass straw silages in the current trial added calcium to the low inherent content of this feedstuff and the consequent prolongation of silage fermentation appeared of benefit for IVDMD of the material not subjected to alkali treatment.

#### Trial IV

Some characteristics of rye grass straw ensiled for 0 to 64 days, with either water + 20% molasses + 1% urea and .5% limestone or 4.5% hydroxide in solution + 20% molasses + 1% urea and .5%

limestone, are presented in table 10. The pH values for silages treated with hydroxide were consistently higher ( $P < .01$ ) than untreated material at each day of observation. The initial pH of 5.9 in untreated silage material dropped ( $P < .01$ ) to 4.9 at 2 days and decreased further ( $P < .01$ ) to 4.4 and 4.3 with 4, 8 and 16 days fermentation. The pH then stabilized at 3.8 following 32 and 64 days ensilation. The hydroxide-treated silages commenced with an 11.6 initial pH, dropped progressively ( $P < .01$ ) to pH 11.1, 6.9 and 4.8 following 2, 4, and 8 days ensilation, respectively, then remained relatively constant from 8 to 64 days.

Silage dry matter was not appreciably affected by materials ensiled or days of ensilation in these mini-silos. The untreated silage dry matter contained lower ( $P < .01$ ) ADF and CWC values at 16 days ensilation while these fractions were highest ( $P < .01$ ) after 64 days fermentation. The proportion of ADL in the untreated dry matter was higher ( $P < .01$ ) at 8 and 16 days ensiling when compared to 0-4 days ensilage. A further increment ( $P < .01$ ) was observed in the 32-day material while the highest ( $P < .01$ ) ADL content was in the 64-day silage. The ADF, ADL and CWC values in the untreated silage dry matter indicate cellulose and, possibly, hemicellulose was being utilized by silage microbes while the increasing values at the later days ensilation may reflect proportionately more lignin fraction consequently remaining in these samples. A similar

Table 10. Characteristics of Ryegrass Straw Silage Ensiled for 0 to 64 days.<sup>a</sup>

Characteristics	Silage type	Days ensiled						
		0	2	4	8	16	32	64
Silage pH	Untreated <sup>b</sup>	5.9 <sup>g</sup>	4.9 <sup>f</sup>	4.4 <sup>e</sup>	4.4 <sup>e</sup>	4.3 <sup>e</sup>	3.8 <sup>d</sup>	3.8 <sup>d</sup>
	Treated <sup>c</sup>	11.6 <sup>i</sup>	11.1 <sup>h</sup>	6.9 <sup>g</sup>	4.8 <sup>f</sup>	4.9 <sup>f</sup>	4.7 <sup>f</sup>	4.9 <sup>f</sup>
Silage dry matter, %	Untreated	23.4	23.6	22.1	22.0	22.5	23.8	22.9
	Treated	23.7	23.9	23.9	22.6	23.7	23.9	22.7
<u>% of dry matter</u>								
Acid detergent fiber	Untreated	41.2 <sup>ef</sup>	40.1 <sup>ef</sup>	39.5 <sup>ef</sup>	40.8 <sup>ef</sup>	38.0 <sup>e</sup>	41.1 <sup>ef</sup>	42.6 <sup>f</sup>
	Treated	39.8 <sup>ef</sup>	38.4 <sup>e</sup>	38.6 <sup>e</sup>	39.5 <sup>ef</sup>	38.6 <sup>e</sup>	42.8 <sup>f</sup>	42.8 <sup>f</sup>
Acid detergent lignin	Untreated	6.1 <sup>e</sup>	6.2 <sup>e</sup>	6.1 <sup>e</sup>	7.1 <sup>f</sup>	7.2 <sup>f</sup>	7.8 <sup>g</sup>	8.4 <sup>h</sup>
	Treated	6.2 <sup>e</sup>	6.0 <sup>e</sup>	6.0 <sup>e</sup>	7.1 <sup>f</sup>	7.9 <sup>g</sup>	8.7 <sup>h</sup>	8.5 <sup>h</sup>
Residual ash	Untreated	3.3 <sup>f</sup>	2.8 <sup>ef</sup>	3.2 <sup>f</sup>	2.8 <sup>ef</sup>	2.4 <sup>e</sup>	1.2 <sup>d</sup>	1.3 <sup>d</sup>
	Treated	3.2 <sup>f</sup>	2.3 <sup>e</sup>	2.2 <sup>e</sup>	2.2 <sup>e</sup>	2.0 <sup>e</sup>	1.1 <sup>d</sup>	1.1 <sup>d</sup>
Cell wall constituents	Untreated	61.4 <sup>ef</sup>	61.6 <sup>ef</sup>	61.4 <sup>ef</sup>	61.7 <sup>ef</sup>	59.7 <sup>e</sup>	62.4 <sup>ef</sup>	66.6 <sup>f</sup>
	Treated	61.6 <sup>ef</sup>	59.0 <sup>e</sup>	58.8 <sup>e</sup>	59.0 <sup>e</sup>	58.1 <sup>e</sup>	61.4 <sup>ef</sup>	65.0 <sup>f</sup>
Crude protein	Untreated	6.5 <sup>f</sup>	6.3 <sup>f</sup>	6.3 <sup>f</sup>	6.8 <sup>f</sup>	6.8 <sup>f</sup>	6.2 <sup>f</sup>	6.6 <sup>f</sup>
	Treated	6.3 <sup>ef</sup>	5.6 <sup>e</sup>	6.0 <sup>ef</sup>	6.3 <sup>ef</sup>	6.3 <sup>ef</sup>	6.2 <sup>ef</sup>	6.5 <sup>f</sup>

<sup>a</sup> Means based on three mini-silos/treatment with two samples/silo

<sup>b</sup> Straw + water + 20% molasses + 1% urea + .5% limestone

<sup>c</sup> Straw + 4.5% hydroxide in solution + 20% molasses + 1% urea + .5% limestone

<sup>d, e, f, g, h</sup> Means in same row or column for each characteristic with unlike superscripts differ significantly (P < .01)

pattern was observed in the hydroxide-treated silage except that the lower ( $P < .01$ ) ADF and CWC values were evident earlier ( $P < .01$ ) with 2-16 days ensilation. Subsequently, increased ( $P < .01$ ) ADL values in this material was observed sooner ( $P < .01$ ) at 16 days ensiling and the remaining lignin component was larger ( $P < .01$ ) in the 32-day sample than that of the untreated material.

Residual ash values were lower ( $P < .01$ ) at 32 and 64 days fermentation for either type of silage as compared to the 0-16 day ensilings. Comparative residual ash was lower ( $P < .01$ ) in the hydroxide-treated silage at 4 days ensiling than untreated material. Crude protein values were not significantly altered during the 0-64 days ensilation of these silages except in the hydroxide-treated material after 2 days fermentation.

From the series of pH values in the untreated silage it is apparent there was sufficient fermentative acidification to neutralize the urea and limestone additives during the extended ensilation period. The buffering capacity of the additives in the hydroxide treated silage material maintained an elevated pH during the ensiling period above that which permits fermentation to subside (Langston et al., 1962; Dexter, 1966; Shirley et al., 1972). Short-term ensiling pH values have not been reported for alkali-treated silages (Koers, Prokop and Klopfenstein, 1972; Klopfenstein et al., 1972), while a marked acidic pH and limited fermentation

resulted with sodium chlorite-treated silages (Goering et al., 1972).

Reports have indicated no change in dry matter content of hydroxide-treated corn stover silage while a reduction in CWC, but not ADF or ADL, occurred (Buchanan-Smith et al., 1972; Klopfenstein et al., 1972). A reduction in lignin content has been observed with hydroxide and/or peroxide-treated corn cob material (Klopfenstein et al., 1972), while decreases of both lignin and hemicellulose fractions have been noted in various crop residues ensiled with sodium chlorite (Goering et al., 1972). These reports and that of the current trial indicate that chlorite appears to act more specifically upon lignin than hydroxide. The apparent lowering of residual ash values in the present trial are not preceded in the literature. A relationship between silica compounds and cell wall components have been indicated in non-ensiled materials (Archibald, 1924; Van Soest and Lovelace, 1969).

Organic acids, expressed as percent of dry matter, from rye-grass straw silage ensiled for 0 to 64 days as either untreated with water + 20% molasses + 1% urea and .5% limestone or treated with 4.5 % hydroxide in solution + 20% molasses + 1% urea and .5% limestone are presented in table 11.

Untreated silage produced an increase ( $P < .01$ ) in lactic acid content with each increment of days ensiled, except between days

Table 11. Organic Acids in Ryegrass Straw Silage Ensiled for 0 to 64 days<sup>a</sup>

Organic acids, % of dry matter	Silage type	Days ensiled						
		0	2	4	8	16	32	64
Lactic acid	Untreated <sup>b</sup>	0.5 <sup>d</sup>	2.1 <sup>f</sup>	3.1 <sup>g</sup>	3.4 <sup>h</sup>	4.8 <sup>i</sup>	5.1 <sup>i</sup>	6.3 <sup>j</sup>
	Treated <sup>c</sup>	0.6 <sup>d</sup>	2.6 <sup>e</sup>	4.0 <sup>f</sup>	5.4 <sup>g</sup>	6.4 <sup>h</sup>	5.6 <sup>g</sup>	3.0 <sup>f</sup>
Acetic acid	Untreated	1.1 <sup>d</sup>	6.1 <sup>e</sup>	7.3 <sup>ef</sup>	6.9 <sup>ef</sup>	7.4 <sup>ef</sup>	8.5 <sup>f</sup>	5.9 <sup>e</sup>
	Treated	3.0 <sup>d</sup>	7.5 <sup>e</sup>	8.5 <sup>eg</sup>	8.8 <sup>eg</sup>	10.6 <sup>g</sup>	12.6 <sup>g</sup>	10.6 <sup>g</sup>
Propionic acid	Untreated	0.8 <sup>d</sup>	0.5 <sup>d</sup>	---	---	---	---	---
	Treated	0.6 <sup>d</sup>	1.4 <sup>e</sup>	1.4 <sup>e</sup>	---	---	0.9 <sup>d</sup>	1.1 <sup>e</sup>
Butyric acid	Untreated	---	2.3 <sup>fg</sup>	1.5 <sup>fg</sup>	2.6 <sup>g</sup>	1.4 <sup>fg</sup>	1.2 <sup>f</sup>	1.4 <sup>fg</sup>
	Treated	---	---	---	---	3.3 <sup>d</sup>	12.1 <sup>e</sup>	11.8 <sup>e</sup>

<sup>a</sup> Means based on three mini-silos/treatment with two samples/silo

<sup>b</sup> Straw + water + 20% molasses + 1% urea + .5% limestone

<sup>c</sup> Straw + 4.5% hydroxide in solution + 20% molasses + 1% urea + .5% limestone

<sup>d, e, f, g, h, i, j</sup> Means in same row or column for each acid with unlike superscripts differ significantly ( $P < .01$ )

16 and 32. The highest ( $P < .01$ ) lactic acid level of 6.3% was observed after 64 days ensilation, indicating a prolonged steady fermentation. Acetic acid formation was essentially completed with a peak value ( $P < .01$ ) of 8.5% at 32 ensilation. Acetic concentration then fell ( $P < .01$ ) to 5.9% at 64 days, which was similar to the 6.1% recorded at 2 days fermentation. Detectable amounts of propionic acid were found only at 0 and 2 days ensiling of this type silage. Butyric acid concentration was too low to measure at day 0 but increased ( $P < .01$ ) to a high of 2.6% after 8 days fermentation, thereafter declining ( $P < .01$ ) to 1.2% at 32 days and remained nearly as such throughout 64 days ensilation.

Hydroxide-treated silage gave a comparatively more rapid increase ( $P < .01$ ) in lactic acid concentration than the untreated silage through 16 days of fermentation. The 6.4% value at 16 days ensilation was lowered ( $P < .01$ ) to 5.6% at 32 days and declined further ( $P < .01$ ) to 3.9% at 64 days ensilation. This 64-day value was lower ( $P < .01$ ) than the respective concentration for untreated silage. Acetic acid levels were higher ( $P < .01$ ) in treated silage during 2 to 64 days fermentation with a peak of 12.6% at 32 days ( $P < .01$ ) compared to 7.5% at 2 days ensilation. Propionic acid in this silage rose ( $P < .01$ ) at 2 and 4 days fermentation, followed by undetectable concentrations at 8 and 16 days ensilation, thereupon a second rise ( $P < .01$ ) was evident at 32 and 64 days after ensiling. Levels of



propionic acid were higher ( $P < .01$ ) in the hydroxide-treated than the untreated silage at 2, 4, 32 and 64 days ensilation. Butyric acid was not measurable until 16 days fermentation elapsed in treated silage. This was followed by elevated ( $P < .01$ ) levels at 32 and 64 days ensilation. The levels of butyric acid in treated silage were markedly higher ( $P < .01$ ) than respective concentrations in untreated silage observed at 16, 32 and 64 days of fermentation.

Descriptions of organic acid production in green plant silages are numerous, while relatively little information is available regarding the ensilation of low quality roughages. Sodium chlorite treatment of straw silage has resulted in marked reductions of organic acids (Goering et al., 1973). The ensiling of late maturity stage bird-resistant sorghum and urea-limestone additives has resulted in low lactic acid production and undesirably high pH values (Johnson, DeFaria and McClure, 1971). Ensiling molasses and urea with corn stover for 90 days increased acetic levels appreciably, but not other acids (Colenbrander, Muller and Cunningham, 1971). The incorporation of 4% hydroxide with corn stover for 50 days ensilation (Buchanan-Smith et al., 1972) resulted in a similar lactic acid value to that observed in the hydroxide-treated silage of the current trial at 32 days fermentation. The previous report indicated a higher pH, yet considerably lower acetic and minimal propionic or butyric acid levels in contrast to the higher acid levels

of the current trial. These comparative results indicate a variation in fermentative patterns influenced possibly by the diverse additives and/or roughage materials used in these trials.

Detailed information (Dijkstra, 1952; Barnett, 1954; Wieringa, 1960; Langston et al., 1962; Langston, Bouma and Conner, 1962; Bousset et al., 1972) indicates that lactic acid is mainly formed by gram-positive lactobacilli, which utilize the more soluble carbohydrates from the silage plant material and/or additives such as molasses. The gram-negative streptococci bacteria produce primarily acetic acid and, secondly, lactic acid by utilizing the more complex plant carbohydrates as they become available during fermentation. Silages having a pH above 4.5 for a prolonged time have been characterized by increasing numbers of sporeforming clostridia bacteria which convert the preformed lactic acid to butyric acid. Relatively small amounts of propionic acid are found in silages, and the significance is not clear but has been associated with the onset of a fermentation pattern when either increased lactic or butyric acid formation occurs (Barnett, 1954).

The characteristics of the present trial suggest a more balanced proliferation of lactate and acetate-forming bacteria occurred in the untreated straw silage while a larger proportion of acetate formers and a replacement of lactic by butyric acid-forming microbes occurred in the hydroxide-treated silage during the

prolonged fermentation period.

The IVDMD and volatile fatty acids produced during the 24 hr in vitro fermentation of ryegrass straw silages are presented in table 12. The silage substrates had been ensiled for 0 to 64 days as either untreated with water + 20% molasses + 1% urea and .5% limestone or treated with 4.5% hydroxide in solution + 20% molasses + 1% urea and .5% limestone.

The IVDMD of untreated silage substrate was unchanged until that from 32 or 64 days ensilation was subjected to in vitro fermentation ( $P < .01$ ). An increased ( $P < .01$ ) IVDMD of treated silage was observed from material ensiled for 2 days, thereafter only minor increments occurred from silage fermented up to 64 days. The IVDMD for treated material was higher ( $P < .01$ ) than untreated silage from day 2 through day 64 of ensiling time. Concentrations of acetic acid were essentially unchanged in the in vitro ruminal fluid of untreated material regardless of previous time in the silo. Acetate increased ( $P < .01$ ) from fermentation of the treated silage having 2 days ensilation. This level and subsequent levels for treated material having up to 64 days ensilation were higher ( $P < .01$ ) than untreated straw ensilage, reflecting the IVDMD values of these silages.

Propionic acid was at a higher ( $P < .01$ ) level following in vitro fermentation of untreated silage having 16 days prior ensiling than

Table 12. Dry Matter Digestion and Volatile Fatty Acids Produced from 24 hr In Vitro Fermentation of Ryegrass Straw Silages Ensiled 0 to 64 Days<sup>a</sup>

Item	Silage type	Days ensiled						
		0	2	4	8	16	32	64
IVDMD, % <sup>b</sup>	Untreated <sup>c</sup>	40.8 <sup>e</sup>	42.1 <sup>e</sup>	42.8 <sup>e</sup>	42.3 <sup>e</sup>	42.9 <sup>e</sup>	46.8 <sup>f</sup>	47.0 <sup>f</sup>
	Treated <sup>d</sup>	41.9 <sup>e</sup>	52.6 <sup>g</sup>	52.0 <sup>g</sup>	54.3 <sup>g</sup>	53.8 <sup>g</sup>	54.5 <sup>g</sup>	54.9 <sup>g</sup>
Acetic acid, mM/1	Untreated	39.2 <sup>e</sup>	40.6 <sup>e</sup>	39.9 <sup>e</sup>	39.7 <sup>e</sup>	41.3 <sup>e</sup>	43.2 <sup>e</sup>	42.5 <sup>e</sup>
	Treated	41.9 <sup>e</sup>	54.8 <sup>f</sup>	51.8 <sup>f</sup>	54.1 <sup>f</sup>	52.0 <sup>f</sup>	55.3 <sup>f</sup>	53.9 <sup>f</sup>
Propionic acid, mM/1	Untreated	27.0 <sup>e</sup>	27.5 <sup>e</sup>	27.3 <sup>e</sup>	29.5 <sup>ef</sup>	32.5 <sup>f</sup>	30.5 <sup>ef</sup>	29.5 <sup>ef</sup>
	Treated	28.8 <sup>ef</sup>	30.4 <sup>ef</sup>	32.2 <sup>fg</sup>	34.5 <sup>g</sup>	34.5 <sup>fg</sup>	28.6 <sup>ef</sup>	28.3 <sup>ef</sup>
Butyric acid, mM/1	Untreated	8.1 <sup>f</sup>	7.7 <sup>f</sup>	6.8 <sup>e</sup>	6.3 <sup>e</sup>	6.8 <sup>e</sup>	6.9 <sup>e</sup>	6.9 <sup>e</sup>
	Treated	8.0 <sup>f</sup>	8.1 <sup>f</sup>	7.9 <sup>f</sup>	7.5 <sup>f</sup>	7.9 <sup>f</sup>	10.2 <sup>h</sup>	14.5 <sup>i</sup>

<sup>a</sup> Means based on three mini-silos/treatment and two samples/silo

<sup>b</sup> Native straw had 32.9% IVDMD

<sup>c</sup> Straw + water + 20% molasses + 1% urea + .5% limestone

<sup>d</sup> Straw + 4.5% hydroxide in solution + 20% molasses + 1% urea + .5% limestone

<sup>e, f, g, h, i</sup> Means in same row or column for each item with unlike superscripts differ significantly ( $P < .01$ )

with this substrate having 2 or 4 days ensilation time. A similar peak was encountered with treated silage having 8 days less ( $P < .01$ ) ensiling time. This value was an increase ( $P < .01$ ) only over the observed value from this type silage having 2 days prior ensilation time. These results indicate more readily fermentable carbohydrates were available at an earlier date from the hydroxide-treated ensilage.

Butyric acid levels were higher ( $P < .01$ ) following in vitro fermentation of untreated silage that had 0 or 2 days ensiling time than this material having been ensiled for longer periods. In contrast, in vitro ruminal fluid butyrate levels were unchanged by treated silage until a marked increase ( $P < .01$ ) occurred with substrate having 32 days ensilation and further increase ( $P < .01$ ) from material having been ensiled for 64 days. These values appear related to the large amount of butyric acid in this silage with corresponding days ensilation (table 11). Butyric acid concentrations were higher ( $P < .01$ ) following fermentation of treated silage than from untreated silage having 4 through 64 days prior ensilation.

The improved IVDMD of hydroxide-treated straw silage conforms with previous in vitro observations in which straw or corn stover silage digestion has been increased with various chemical treatments (Krause, Klopfenstein and Woods, 1968; Yu Yu, Thomas and Emery, 1970; Klopfenstein et al., 1972; Koers, Prokop and

Klopfenstein, 1972; Goering et al., 1973). The apparent IVDMD increase observed in the present trial with untreated silage having prolonged ensilation was not evident in the literature reviewed. This effect has been negligible following shorter term ensilation (Goering et al., 1973).

Available reports do not mention VFA values from in vitro rumen fermentation of substrate similar to that used in the present trial. In vivo studies have shown increased total VFA concentrations in rumen fluid of animals consuming low-quality roughages ensiled with hydroxide (Koers, Woods and Klopfenstein, 1970; Klopfenstein et al., 1972). Feeding chlorine-treated straw silage has increased total VFA and the proportion of propionate (Yu Yu, Thomas and Emery, 1971). In contrast, chlorite-treated straw silage gave decreased rumen total VFA and acetate while increasing propionic and butyric acid levels (Goering et al., 1973). The VFA values reported previously and that observed in the present trial suggest chemical specificity in cell wall degradation processes may occur, but generally increased rumen fermentation products result from the treated substrate.

## Straw Silage Utilization by Animals

### Digestion Trial

Apparent digestion and nitrogen retention by lambs fed rye-grass straw silage, ensiled as either untreated with water + 20% molasses + 1% urea and .5% limestone or treated with 4.5% hydroxide in solution + 20% molasses + 1% urea and .5% limestone, are presented in table 13. Chemical composition and organic acid contents are listed in table 4.

Lambs consumed the hydroxide-treated silage both more readily and rapidly. The treated straw material had a more compact, denser aspect than the untreated straw silage. Although animals apparently consumed the untreated silage more slowly, there were essentially no refusals when offered at a maintenance feeding level. Dry matter intake and, consequently, nitrogen intake were greater ( $P < .05$ ) with the treated silage than the untreated material. Consumption of free-choice tap drinking water and trace mineralized salt was slightly higher by animals receiving treated silage; however, the difference was due primarily to variable abnormal intakes by one lamb throughout the trial.

Apparent digestion of dry matter, organic matter, ADF, ADL CWC and crude protein were all higher ( $P < .05$ ) with the hydroxide-

Table 13. Apparent Digestion and Nitrogen Retention of Ryegrass Straw Silage by Lambs<sup>a</sup>

Item	Silage type	
	Untreated <sup>b</sup>	Treated <sup>c</sup>
Dry matter intake, g/day	710 <sup>d</sup> $\pm$ 94	949 <sup>e</sup> $\pm$ 167
Dry matter digestion, %	46.9 <sup>d</sup> $\pm$ 2.4	60.7 <sup>e</sup> $\pm$ 2.3
Organic matter digestion, %	48.5 <sup>d</sup> $\pm$ 5.1	62.2 <sup>e</sup> $\pm$ 2.2
Acid detergent fiber dig., %	40.4 <sup>d</sup> $\pm$ 5.0	54.3 <sup>e</sup> $\pm$ 2.9
Acid detergent lignin dig., %	1.2 <sup>d</sup> $\pm$ 8.0	11.1 <sup>e</sup> $\pm$ 5.7
Cell wall constituent dig., %	44.9 <sup>d</sup> $\pm$ 4.4	62.5 <sup>e</sup> $\pm$ 3.0
Crude protein digestion, %	43.7 <sup>d</sup> $\pm$ 4.9	52.6 <sup>e</sup> $\pm$ 4.6
<u>Daily values</u>		
Nitrogen intake, g	8.9 <sup>d</sup> $\pm$ 0.9	11.6 <sup>e</sup> $\pm$ 1.8
Fecal nitrogen, g	5.0 <sup>d</sup> $\pm$ 0.4 (56.2)	5.5 <sup>d</sup> $\pm$ 1.1 (47.4)
Urinary nitrogen, g	5.8 <sup>d</sup> $\pm$ 0.7 (65.2)	5.7 <sup>d</sup> $\pm$ 1.5 (49.1)
Retained nitrogen, g	-1.9 <sup>d</sup> $\pm$ 0.8	0.4 <sup>e</sup> $\pm$ 1.7
Urine output, ml	1797 <sup>d</sup> $\pm$ 556	2654 <sup>e</sup> $\pm$ 1406
Urine specific gravity	1.007 <sup>d</sup> $\pm$ .002	1.012 <sup>e</sup> $\pm$ .003
Urine excreted per dry matter feed intake, ml/g	2.5 <sup>d</sup> $\pm$ 0.6	2.8 <sup>d</sup> $\pm$ 1.0

<sup>a</sup> Means  $\pm$  SD based on eight animals/treatment; values in parentheses are percent of intake.

<sup>b</sup> Straw + water + 20% molasses + 1% urea + .5% limestone

<sup>c</sup> Straw + 4.5% hydroxide in solution + 20% molasses + 1% urea + .5% limestone

<sup>d, e</sup> Means in same row with unlike superscripts differ significantly ( $P < .05$ )

treated silage as compared to the untreated ensilage. These results indicate a marked degradation of the structural carbohydrates and solubilization of lignin by the hydroxide additive. The trial IV IVDMD of the untreated silage having 32 or 64 days ensilation are in close proximity to the apparent dry matter digestion by these lambs. An increase of 5% is noted in the in vivo dry matter digestion of the hydroxide-treated straw silage as compared to



trial IV IVDMD for this material having 32 or 64 days ensilation.

Lambs consuming untreated silage retained less ( $P < .05$ ) nitrogen and were in a negative balance of -1.9 g N/day as opposed to animals retaining 0.4 g N/day when fed the treated silage. Expressing the daily fecal nitrogen excretion of 5.0 g from untreated silage and 5.5 g from treated silage as a percent of nitrogen intake reveals 56.2% and 47.4% defecated, respectively. A numerically greater volume of urine/unit of dry matter intake was voided by animals consuming the hydroxide-treated silage and this urine had a higher ( $P < .05$ ) specific gravity. This would be expected due to the elimination of the excess cations from this diet. It is of interest to note that 16% more urinary nitrogen was excreted by animals consuming the untreated silage than those receiving the hydroxide-treated material. Increased urinary ammonium ion excretion would be anticipated due to the highly acidic nature of the untreated silage. This could partially account for the increased urinary nitrogen loss observed.

Improved in vivo digestion of structural components in low-quality roughages has been demonstrated by ensiling with chemicals (Krause, Klopfenstein and Woods, 1968; Kormscikov, 1968; Koers, Klopfenstein and Woods, 1969; Koers, Woods and Klopfenstein, 1970; Yu Yu, Thomas and Emery, 1971; Mowat, 1971; Klopfenstein et al., 1972; Goering et al., 1973). Some of these reports

indicated intake problems even though silage was fed with supplements (Mowat, 1971; Yu Yu, Thomas and Emery, 1971), while the other silages, fed with protein supplements and/or ion neutralization additives, did not present consumption problems.

Hydroxide-treated stover silage, fed as nearly 50% of a ration with alfalfa, demonstrated greater nitrogen retention while not affecting nitrogen digestion (Klopfenstein et al., 1972). Untreated stover silage, fed in similar proportions, resulted in a negative nitrogen balance with only a minor reduction in nitrogen digestion. Animals consuming supplemented hydroxide-treated corn stover and cob silage retained more nitrogen than animals receiving untreated silage although nitrogen digestion was not appreciably affected (Koers, Klopfenstein and Woods, 1969; Koers, Woods and Klopfenstein, 1970). As in the present trial, greater urine volume and higher nitrogen retention have been observed with treated silage diets (Goering et al., 1973). In contrast to these reports, hydroxide-treated sorghum silage resulted in reduced protein digestion and no influence on nitrogen retention (Bolsen, Chyba and Riley, 1972).

Observations on rumen VFA from lambs used in the current digestion trial are listed in table 14. Ruminal fluid from animals receiving hydroxide-treated silage had greater ( $P < .05$ ) concentrations of acetic and propionic acids, while butyric acid was numerically

Table 14. Rumen Volatile Fatty Acids from Lambs Fed Ryegrass Straw Silage<sup>a</sup>

VFA, mM/l	Silage type	
	Untreated <sup>b</sup>	Treated <sup>c</sup>
Acetic	40.1 <sup>d</sup> ± 5.0	58.5 <sup>e</sup> ± 4.8
Propionic	12.8 <sup>d</sup> ± 1.6	18.9 <sup>e</sup> ± 2.6
Butyric	4.0 <sup>d</sup> ± 1.0	7.2 <sup>d</sup> ± 1.3
Total C <sub>2</sub> - C <sub>4</sub>	56.9	84.6
C <sub>2</sub> :C <sub>3</sub>	3.2	3.1

<sup>a</sup> Means ± SD based on eight animals/treatment

<sup>b</sup> Straw + water + 20% molasses + 1% urea and .5% limestone

<sup>c</sup> Straw + 4.5% hydroxide in solution + 20% molasses + 1% urea and .5% limestone

<sup>d, e</sup> Means in same row with unlike superscripts differ significantly (P < .05)

higher, in comparison to values from lambs consuming the untreated silage. Although the total of these volatile fatty acids was of a larger magnitude from the animals on treated silage, the ratio of acetic to propionic acid was similar from lambs consuming either type of silage.

Animals receiving crop residues ensiled with hydroxide have had larger total VFA concentrations in rumen fluid contents when compared to untreated silages (Koers, Woods and Klopfenstein,

1970; Klopfenstein et al., 1972). Rumen fermentation of chlorine-treated straw silage has resulted in a higher proportion of propionate and increased total VFA levels (Yu Yu, Thomas and Emery, 1971), while feeding chlorite-treated silage produced a higher propionate but lowered total VFA in comparison to untreated material (Goering et al., 1973). Diverse influences of silage additives upon fermentation products in the silo and the rumen have been reported (McCullough, 1966; McCullough Sisk and Smart, 1970). Future studies regarding these relationships with specific additives and low-quality roughage ensilage should prove both interesting and worthwhile.

#### Performance Trial

Body weight gains by heifers and consumption of ryegrass silage, untreated and hydroxide-treated ryegrass straw silages supplemented with either a molasses-fat-urea semi-solid mix or a barley-cottonseed meal dry mix are presented in table 15. Silage organic acid content and chemical composition of feedstuffs are listed in table 6.

Animals receiving either of the three silages supplemented with the dry mix gained at a faster ( $P < .05$ ) rate than heifers consuming the semi-solid supplement regardless of the silage offered. It is of interest to note the increased consumption of the straw

Table 15. Body Weight Gains and Consumption of Silages and Supplements by Heifers<sup>a</sup>

Observations	Silage type Supplement	Ryegrass		Untreated straw <sup>b</sup>		Treated straw <sup>b</sup>	
		Semi-solid <sup>c</sup>	Dry <sup>d</sup>	Semi-solid	Dry	Semi-solid	Dry
Initial wt, kg		234	234	236	235	232	232
Final wt, kg		238	280	240	274	227	277
ADG, g		44 <sup>f</sup> ± 66	458 <sup>g</sup> ± 267	43 <sup>f</sup> ± 165	398 <sup>g</sup> ± 202	-52 <sup>f</sup> ± 91	454 <sup>g</sup> ± 229
<u>DM intake, kg/hd/day</u>							
Supplement day 1-60		0.8	0.9	0.8	0.9	0.8	0.9
Supplement day 60-98 <sup>d</sup>		0.4	0.9	0.4	0.9	0.4	0.9
Silage day 1-60		4.8	5.5	2.8	3.4	3.3	3.6
Silage day 60-98		3.5	4.3	4.8	5.1	4.6	5.2
Silage day 1-98		4.2	4.4	3.7	4.2	3.8	4.4

<sup>a</sup> Means ± SD based on five animals /treatment.

<sup>b</sup> Described in table 14

<sup>c, d</sup> Described in tables 5 and 6

<sup>e</sup> Day 60-98 all animals, additional .9 kg/hd/day fattening supplement, described in tables 5 and 6

<sup>f, g</sup> Means in same row with unlike superscripts differ significantly (P< .05)

silages and an apparent decrease of ryegrass silage intake during days 60-98 when the .9 kg fattening supplement was given in addition to other supplements. Overall silage intake for ryegrass or hydroxide-treated straw silages were similar while untreated straw silage consumption was numerically less. The straw silages were adequately conserved by the additives used and only minor top spoilage occurred. Noticeable effluent loss from silos containing straw, due to faulty closure at the base, may have detracted from silage quality somewhat.

The ensiling of crop residues with additives such as urea and hydroxide, and supplemented in similar proportion as the present trial, have generally improved animal performance when compared to untreated or non-ensiled material (Koers, Klopfenstein and Woods, 1969; Koers, Woods and Klopfenstein, 1970; Colenbrander et al., 1971; Koers, Prokop and Klopfenstein, 1972). The addition of hydroxide to whole sorghum or corn plants at ensiling have not appreciably affected animal responses (Krause, Klopfenstein and Woods, 1968; Bolsen, Chyba and Riley, 1972). The narrowed differences of animal performance observed in the current trial may be due in part to the types and amounts of additives used in the silages as well as supplementation effects.

The depressed silage intakes observed with the semi-solid supplement may be attributed to the molasses and the fat. Both

ingredients have been shown to lower digestion and intake of roughages while the addition of nitrogen and calcium may alleviate the situation (Loosli and McDonald, 1969; Johnson and McClure, 1973). Intake of the semi-solid mix was voluntarily reduced by one-half when the additional fattening supplement was offered during the final phase of this trial. The subsequent stimulated intake of both types of straw silage did not occur with the ryegrass silage. A recent report has demonstrated depressed utilization of grass silage by both sucrose and starch supplements but not by added cellulose (Syrjala, 1972).

The relatively higher pH and butyrate content of hydroxide-treated straw silage apparently did not affect intake. This observation is supported by earlier reports (Gordon et al., 1959; McLeod, Wilkins and Raymond, 1970), although increased butyric acid in silage has been associated with reduced intake (Harris, Raymond and Wilson, 1966; McCullough, Sisk and Smart, 1970). The essentiality of the additives ensiled with the untreated straw seemed evident in promoting a desirable fermentation and palatability of this feedstuff. This is substantiated by the recent reports of others ensiling a highly fibrous material (O'Donovan, Chen and Lee, 1972).

## SUMMARY

A series of experiments utilizing laboratory silos and in vitro digestion, followed by animal metabolism and performance trials, were used to evaluate the effects of various additives ensiled with annual ryegrass straw.

Straw ensiled with water or 4.5% NaOH:KOH in solution presented undesirably high pH ( $P < .01$ ) values while .5 g cellulase in water gave a lower pH ( $P < .01$ ). The inclusion of 10% and 20% molasses in these silages lowered pH ( $P < .01$ ) concomitantly. Straw ensiled with only water retained the least ( $P < .01$ ) crude protein. IVDMD was most improved ( $P < .01$ ) with hydroxide silage while enzyme addition gave an improvement ( $P < .01$ ) over straw and water ensilage. Increases ( $P < .01$ ) in the IVDMD of silages having molasses corresponded to the increments of molasses used.

The addition of 1% urea or 1.2% biuret increased ( $P < .01$ ) crude protein content in straw ensiled with water and 20% molasses or 4.5% hydroxide and 20% molasses. IVDMD of these silages were increased ( $P < .05$ ) by addition of urea but not with biuret. Adding urea to the water and molasses increased ( $P < .01$ ) silage pH but not in the hydroxide-treated material. Incorporating .1% elemental sulfur to silages without NPN raised ( $P < .01$ ) pH. Combining sulfur in hydroxide silages resulted in some spoilage, particularly that



having urea. A decrease ( $P < .01$ ) in ADF and CWC resulted from the sulfur-biuret additives to hydroxide silage.

Ensiling straw with water, 20% molasses and 1% urea for 40 days lowered ( $P < .01$ ) silage pH as compared to 20 days ensilation. The addition of .5% limestone furthered ( $P < .01$ ) this effect. Hydroxide-treated silage having 20% molasses and 1% urea resulted in the highest ( $P < .01$ ) pH while limestone reduced ( $P < .01$ ) this effect. Adding .5% formic acid gave lowered ( $P < .01$ ) pH values in these ensilages. IVDMD was improved ( $P < .01$ ) after 40 days ensilation by adding limestone to molasses-urea silage. Residual ash values were reduced ( $P < .01$ ) in 40 day silages as compared to 20 days fermentation regardless of additives.

During 0 to 64 days ensilation, pH values were consistently higher ( $P < .01$ ) in hydroxide-treated silage containing 20% molasses, 1% urea and .5% limestone. Decreases ( $P < .01$ ) in ADF and CWC, and an increase ( $P < .01$ ) in ADL occurred during the 64 days ensiling of the untreated silage. Similar trends resulted several days earlier ( $P < .01$ ) in the hydroxide-treated ensilage. Residual ash values were lower ( $P < .01$ ) in either type silage after 32 days ensilation. Untreated silage presented a higher ( $P < .01$ ) lactic acid concentration at 64 days while acetic acid peak ( $P < .01$ ) value was at 32 days. This type silage had essentially no propionic acid after 2 days ensilation, while butyric acid was higher ( $P < .01$ ) at 8 days

and lowest ( $P < .01$ ) at 32 days. Hydroxide-treated silage had peak ( $P < .01$ ) lactic acid at 16 days ensilation, then dropped ( $P < .01$ ) thereafter. Acetic acid in this silage plateaued ( $P < .01$ ) at 16 days, while butyric acid was undetectable until 16 days, thereupon rising ( $P < .01$ ) sharply. Rises ( $P < .01$ ) in propionic acid were observed at 2 and 32 days in this silage.

The IVDMD for untreated silage rose ( $P < .01$ ) for material having been ensiled for 32 days, while hydroxide-treated ensilage was more ( $P < .01$ ) digestible with 2 or more days ensilation. A similar pattern was evident for in vitro rumen liquor acetic acid levels and, to a lesser extent, for propionic acid. These fluids showed more ( $P < .01$ ) butyric acid from hydroxide-treated substrate than the untreated.

Lambs consumed more ( $P < .05$ ) hydroxide-treated silage than untreated and had higher ( $P < .05$ ) digestion of dry and organic matter, ADF, ADL, CWC and crude protein. Nitrogen intake and retention was higher ( $P < .05$ ) from treated silage. Rumen acetate and propionate concentrations were greater ( $P < .05$ ) from hydroxide-treated silage, while the ratio of these were similar to that from untreated ensilage fed.

Heifers fed .9 kg/hd/day barley-cottonseed supplement gained similarly while receiving either ryegrass silage or untreated or hydroxide-treated straw silages. Animals offered the same silage

with a fat-molasses-urea supplement all gained less ( $P < .05$ ).

Untreated straw silage intake was slightly lower than other silages regardless of supplement given. Intake of both straw silages was stimulated while that of ryegrass silage decreased when .9 kg/hd/day additional fattening supplement was offered during the final 38 days of the trial.

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