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Preservative Effects of Covering and Propionic Acid of Alfalfa Haylage in Bunker Silos

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PRESERVATIVE EFFECTS OF COVERING AND PROPIONIC ACID ON ALFALFA HAYLAGE IN BUNKER SILOS

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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I dedicate this thesis to my wife, Kristy, for her patience, love, encouragement, and hours spent alone.

TJO

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ABSTRACT

The preservation efficiency of covering alfalfa haylage with black plastic (polyethylene) and/or treating haylage with propionic acid was studied in two trials. Experiment 1 was designed to evaluate the influence of both covering and treatment with propionic acid on haylage chemical composition and heifer growth. In experiment 1, propionic acid was administered to the haylage at the chopper at 0.02% of the fresh forage weight. Chemical composition and ensiling temperature of the haylage were monitored and animal growth was measured with 16 Holstein heifers. Covered haylage was superior to treated haylage in quality as measured by chemical analyses and animal performance. Propionic acid lowered ensiling temperature to a lesser extent than covering. Experiment 2 was designed to compare a control alfalfa haylage (covered/untreated) to an uncovered haylage topically treated with 100% propionic acid. Ensiling temperature, chemical content, and animal performance of dairy heifers were evaluated. The control haylage had lower ensiling temperature and was superior in quality as measured by chemical analyses and heifer performance. Propionic acid addition was ineffective in lowering ensiling temperature and limiting extended fermentation. The data suggests that covering was more efficient than propionic acid addition in preserving alfalfa haylage in bunker silos.

TABLE OF CONTENTS

TABLE OF CONTENTS
(continued)

TABLE OF CONTENTS
(continued)

TABLE OF CONTENTS
(continued)

TABLE OF CONTENTS
(continued)

LIST OF TABLES

LIST OF TABLES
(continued)

LIST OF FIGURES

LIST OF APPENDIX TABLE

LIST OF APPENDIX FIGURES

INTRODUCTION

Alfalfa is commonly grown in the Midwest and is a staple in many dairy rations. Wilting alfalfa haylage to 40% to 60% moisture (44) and storing in bunker silos prior to feeding is a desirable technique for preserving legume forages (76). Due to the large surface area exposed to oxygen in bunker silos, haylage may undergo severe heating, heat-damaged protein loss, storage losses, and molding (110). Heat damage of haylage may be observed more often in low-moisture haylage than in haylage with high moisture levels. Covering bunker silos should reduce air exposure to the silage resulting in a superior fermentation. The addition of propionic acid to haylage to reduce temperature and mold has been well documented (110, 126).

It was the intent of this investigation to test the preservative values of covering and/or treatment with propionic acid. Efficacy of various preservative methods were evaluated by measuring haylage chemical composition and animal performance. Addition of propionic acid in experiment 1 was throughout the entire haylage mass while that in experiment 2 was topically treated.

LITERATURE REVIEW

Terminology

A silo is a structure, usually a cylindrical pit or tower, in which fodder, grains, or other food is stored green to be fed at a later date to cattle. Silage is the feedstuff resulting from the anaerobic preservation of moist feedstuffs by the formation and/or additions of acids (68). Other terms such as haylage, cornlage, oatlage, and animal waste silage are terms describing an ensiling process (68). Silage is divided into three groups based on moisture level. These groups are high-moisture or direct-cut silage (70% + moisture), wilted silage (60 to 70% moisture), and low-moisture silage (40 to 60% moisture) (81).

Factors Affecting Silage Utilization

Ensiling is a process of preserving feed for livestock, and the success of this process is measured in terms of preservation efficiency and endproduct usefulness in animal feed (68).

The primary factor affecting animal performance is the feeding value of the crop at time of ensiling. Two important factors influencing feed value are dry matter intake and dry matter digestibility of silage (68). McCullough (67) found that 89% of the variation in average daily gains of growing dairy heifers was explained by dry matter digestibility of the silage and dry matter intake. Ninety-three percent of the variation in milk production in dairy cows was explained by total digestible nutrients intake, body weight, and percent total digestible nutrients in silage dry matter (DM)

(69). A major factor in silage utilization is stage of maturity at harvest which controls both dry matter digestibility and dry matter intake (68). Demarquilly and Jarrige (25) showed a direct relationship between dry matter digestibility and dry matter intake. Their study, as well as many others, has drawn two conclusions: First, the optimum time for harvest is a compromise between dry matter digestibility per unit of dry matter and total dry matter per acre. Secondly, each plant species will have an optimum stage for harvest depending upon its individual characteristics.

Geographical location and weather affect plant growth as well as suitability for ensiling. Crops grown in hot climates are less digestible than the same crops grown in cool climates (68). Minson and McLead (78) demonstrated a -0.89 correlation between dry matter digestibility and the mean temperature during growth for several grasses cut at monthly intervals. Ambient temperature can also affect the silage fermentation process. Ammonical nitrogen and butyric acid in silages made from the same forage were highest in those forages ensiled at ambient temperatures ranging from 25 to 45^{o}C (118).

In addition to the variables of crops and weather, harvesting and storing operations may also affect the feeding value of the silage. Technology, additives, and aeration are the other variables affecting silage utilization (131). Technology in silage production includes wilting, chopping length, and filling rate of the silo. The purpose of wilting is to increase the dry matter content of the

forage to be ensiled, to concentrate fermentable carbohydrates, and to reduce seepage (68). Length of chop is correlated with the following factors: 1) density of packing in the silo, 2) efficiency of fermentation, 3) intake of silage, and 4) amount of seepage. In general, cutting the forage in shorter lengths increases density of packing, decreases energy loss, and increases silage intake. The optimum length of cut is 1.5 cm clearance in the chopper (29, 130). Cutting length becomes more critical as the dry matter content increases (67). Miller et al. (77) showed that a faster ensiling rate decreased losses for dry matter, protein, nitrogen-free extract, and ash. Silage ensiled slowly had a higher peak temperature that persisted longer and had a lower lactic acid value than the other silage (77).

Sizeable losses in silage preservation and quality are associated with aeration. Aeration losses are increased with prolonged wilting, slowed filling, delayed covering, and cracked silo walls. Aeration prolongs the development of anaerobic conditions and the beginning of lactic acid fermentation and causes depletion of fermentable carbohydrates and degradation of proteins (68). Silage additives will be discussed later in this paper.

Evaluation of Silage Fermentation

"Silage quality" is generally used to indicate the success of the fermentation and not the feeding value of the silage. Quality silage production depends upon highly digestible nutrients to support fermentation; however, poor fermentation reduces the feeding value

of the silage. Therefore, silage quality and the nutritional value of the silage are highly correlated (68). To measure silage quality certain parameters are used. Gordon et al. (45) correlated seven chemical fractions of silage to dry matter intake of dairy cows. Dry matter content of the crop and percent lactic acid formed during fermentation were positively correlated to dry matter intake (45). Percent butyric, propionic, and acetic acids in the silage and silage pH were negatively correlated to dry matter intake (45). Multiple regression analysis indicated that 64% of the variation in dry matter intake was explained by percent dry matter, butyric acid, and lactic acid (45). McCullough (66), using lactating dairy cows, indicated that crude protein percentage influenced silage dry matter intake and that crude protein was an indicator of plant maturity. Breiren and Ulvesli (16) used the measures in Table 1 for good silage fermentation. Nilsson et al. (80) developed five

TABLE 1. Levels of the factors used for quantifying proper silage fermentation.

Criteria	Values
pH	4.2 (maximum)
Lactic acid (%)	1.5 to 2.5
Acetic acid (%)	0.5 to 0.8
Butyric acid (%)	below 0.1
Ammonical-N in % of total N	not above 5 to 8

silage quality groups (very good to very bad) based on butyric acid and ammonical nitrogen contents. Silage with a butyric acid content less than 0.10 (% DM) is very good silage and silage with a content greater than 0.40 (% DM) is very bad. Ammonical nitrogen levels of less than 12.5% of total nitrogen (TN) and greater than 20.1 (% TN) in silage corresponds to very good and very bad qualities, respectively. A method commonly used for evaluating silage quality has been the system using Fleig points (34). Although modified (128), these points were based upon the percent of lactic, acetic, and butyric acids in the silage. Fleig scores were significantly correlated to intake and digestibility of the silage (104). The National Feed Ingredients Association lists thirteen criteria used to measure quality of silage (85). These criteria are percent solids, pH, total lactic acid, total energy, residual carbohydrates, total protein, pepsin insoluble nitrogen, acid detergent nitrogen, neutral detergent fiber, ammonia or volatile nitrogen, lignin, volatile fatty acids, and microbiological composition.

Chemistry of Silage Fermentation

Introduction. If silage is exposed to air, microbial activity involving yeasts, fungi, and bacteria takes place resulting in high gaseous losses of dry matter. If silage is under anaerobic conditions, but contains less than 28% dry matter and has a high pH, it is still subject to deterioration of dry matter (30). However, a silage of higher dry matter and/or low pH (lactic acid bacteria in large supply) is quite resistant to anaerobic clostridia. Yeasts are

not a problem under anaerobic conditions, but are present in a dormant stage. They remain inactive until the silo is opened, allowing aerobic conditions and promoting fungal growth and destruction of fermentation acids and residual sugars (30).

Role of microbes in silage fermentation. Aerobic microbes are the most numerous on fresh forage, but Escherichia, Klebsiella, Bacillus, Streptococcus, Leuconostoc, Lactobacillus, and Pediococcus, also occur. Clostridia are present, but in endospore form (116). Lactic acid producing bacteria are responsible for preserving silage because they produce lactic acid which lowers the pH to 4.2. At a pH of 4.2 all microbial activity or fermentation ceases. Wood (120) has classified lactic acid bacteria into homofermentative and heterofermentative types. These types differ in their end-products of fermentation and their efficiency to produce lactate. Appendix Table 1 lists lactic acid bacteria commonly found in silage (30).

Role of carbohydrates in silage fermentation. Glucose, fructose, and sucrose are the main sugars of herbage (72). There are traces of melibiose, raffinose, stachyose, mannoheptulose, D-glycero-D-manno-octulose, fructosylfuranose, and fructosylglucose in a variety of plant species.

Fructans and starches are the main storage carbohydrates of grasses and legumes (30). These non-structural carbohydrates are hydrolyzed by plant enzymes into their constituent monomers (116). The monomers, glucose and fructose, are chief substrates for the micro-organisms during ensilage (116). Hemicellulose is a structural

carbohydrate in the plant and the only structural carbohydrate subject to microbial fermentation. During silage fermentation it is broken down to arabinose and xylose (27, 104).

When a forage is ensiled, plant enzymes break the non-structural carbohydrates down to simple sugars. The sugars are the substrate medium for the fermentative bacteria. After several hours of storage, anaerobiosis occurs. The breakdown of sugars may be accomplished by the homofermentative or heterofermentative lactic acid bacteria depending on their predominance (30). The glycolytic pathway is the preferred mechanism in homolactic fermentation. Heterolactic fermentation prefers the hexose monophosphate pathway (120). Appendix Figures 1 through 4 are the major pathways of lactic acid bacteria (30).

The role of organic acids in silage. Fauconneau and Jarrige (32) reported levels of organic acids between 20 and 60% of dry matter in grasses and 60 to 80% of dry matter in legumes. Malate and citrate were the major acids of ryegrass; malate and glycerate were the major acids in fresh red clover (87). Within the pH range 4 to 6, the organic acids were responsible for 68 to 80% of the total buffering power of the herbage (30). As the herbage wilts, the buffering power declines because of the loss of organic acids (86).

The organic acids are broken down either by plant enzymes or bacteria. This results in an initial loss of buffering capacity and a rise in pH (53, 87, 115). As fermentation continues, pH lowers

and lactic and acetic acids increase until fermentation ceases (30). Appendix Figure 5 is an overview of organic acid fermentation by lactic acid bacteria (30, 74).

The role of nitrogenous constituents in silage. Protein makes up 75 to 90% of the total nitrogen in fresh herbage. The remainder is non-protein nitrogen, consisting mainly of free amino acids, glutamine and asparagine, amines, ureides, and low molecular weight peptides (52). Ammonical nitrogen levels are less than 1.0 to 1.5% of total nitrogen in fresh forage (12, 14, 72). Nitrate nitrogen occurs at variable levels in herbage (30). Several researchers have reported that the amino acid composition of protein among several groups of plant species was similar (130).

Plant enzymes in the first 5 days of fermentation cause proteolysis as evidenced by increases in water-soluble nitrogen and non-protein nitrogen. As the pH lowers to 4.3, proteolysis ceases (30). Certain amino acids disappear during ensiling (108). Lactic acid bacteria are capable of decarboxylating tyrosine, histidine, lysine, and ornithine (30, 35, 91, 92). Lactobacillus plantarum and Pediococcus species deaminate serine to pyruvate and arginine to ornithine. Lactobacillus brevis deaminates arginine, glutamine, and asparagine (15).

Ammonical nitrogen levels in good quality silage are often 9 to 11% of total nitrogen (70, 75). Ammonia is the result of deamination by clostridia (38, 63) and/or is the result of nitrate reduction (121).

Clostridia during silage production. If the lactic acid bacteria do not lower the pH of the silage quickly and if the ensiled material is too wet, clostridial bacteria will grow (116, 117). Clostridia are of two types, saccharolytic and putrefactive. The saccharolytic clostridia break down hexose and lactate to butyrate (71). (See Appendix Figure 6). Butyric acid has a lower buffering potential than lactic acid; therefore, pH rises providing a favorable medium for putrefactive clostridia (70). These organisms break down amino acids to ammonia in poorly preserved silages (55). Poorly preserved silages are characterised by having high pH, high water-soluble nitrogen content, and high volatile nitrogen content (70).

Fungi in silage production. Deterioration of silage is a major problem upon opening of a silo. Yeasts deteriorate silage by catabolizing fermentation acids and residual sugars to carbon dioxide which is a loss of dry matter (10, 129). Mold produces toxins that will cause diarrhea, irritability, and loss of appetite in calves fed the infested silage (21, 86).

Direct Acidification of Silage

Wilted hay-crop silages are difficult to ensile at an optimum dry matter. Even at optimum dry matter, protein degradation is considerable. If the forage becomes too dry, additional protein becomes indigestible due to heat damage. Untreated direct-cut silages have low recoveries of energy and nitrogen. Lowered intake, partial feed conversion, daily animal production, and animal

production per ton or hectare result (110).

Direct acidification of ensiled hay-crop forages ranks second to wilting for preserving hay-crop forages around the world. The first work with direct acidification was by A. I. Virtanen in 1925 (111). His early studies demonstrated that a pH near four restricted respiration, proteolysis, and secondary or butyric acid fermentation in forages. Virtanen worked primarily with mineral acids. Since 1956, considerable research and on-farm-use has occurred using formic acid-formaldehyde mixture, and sulfuric acid-formaldehyde mixture (111) for silage preservation.

Most work with these acids has occurred in northern Europe, North America, Australia, New Zealand, and Japan. A majority of the temperate grasses, clovers, and alfalfa have been treated. The levels of formic acid used alone have ranged from 0.72 to 3.66% of dry matter (111). When formic acid and formaldehyde are mixed together, formic acid is added at 0.45 to 1.65 (% DM) and formaldehyde is added at 0.36 to 1.5% of dry matter (111). Formaldehyde was used alone at levels ranging 0.36 to 1.8% of dry matter (111). Acids may be sprayed on the standing crop to reduce moisture level, or added at the time of ensiling. Similar rates of application were used on standing crops as well as on the crop as it was ensiled (110). No data on the former method is available (111), but Norgaard-Pedersen et al. (82) stated that application of acid at the silo was better.

Waldo, in his review (111) of silage fermentation, compared

the different acids on the basis of recovery from storage, feeding value, and chemical composition.

Recovery from storage. Formic acid increased the recovery of direct-cut silage by 5% and of wilted silage by 8%. The formic acid-formaldehyde mixture increased dry matter recovery from storage by 1%, and formaldehyde increased dry matter recovery by 5% (111).

Intake. Formic acid increased the digestible energy intake of young cattle by 20% for high moisture silages and 6% for wilted silages. The formic acid-formaldehyde mixture increased intake by 13%, and formaldehyde increased intake by 23%. Formic acid alone or in mixtures with formaldehyde retained nearly all of the potential intake of the original crop (111).

Formic acid increased the dry matter intake of direct-cut silages, given to lactating cows fed supplemental concentrates, by 12% and wilted silages by 9%. The formic acid-formaldehyde mixture increased intake by 13% (111).

Digestibility. The digestibility of metabolizable energy was affected very little by chemical treatments. Digestibility of organic matter was higher for the treated silages except for formaldehyde treated silage (111). Unlike intake, digestibility of silage is affected very little by chemical treatment.

Daily gain. All chemical treatments increased the weight gains obtained from feeding direct-cut silages: formic acid, 71%; formic acid-formaldehyde mixture, 67%; and formaldehyde, 74%. Formic acid increased the gains obtained from feeding wilted silages

by 27% (111).

Milk production. Formic acid increased milk production from cows fed direct-cut silages by 5% and milk production from wilted silage by 2%. The formic acid-formaldehyde mixture increased milk production by 5%, and formaldehyde increased it by 13% (111). Milk production was 6% greater from cows fed formic acid silage than cows fed hay cut at the same time (97). The same researchers found no difference in milk production from cows fed either formic acid silage and dehydrated grass (98). Milk production is not affected as much as weight gain by chemical treatment (111).

Feed efficiency. Formic acid increased feed efficiency by 12% for both direct-cut and wilted silages. The formic acid-formaldehyde mixture decreased feed efficiency by 13% (based on one experiment) (111). Formaldehyde increased feed efficiency by 24% (111).

Weight gain per ton of ensiled forage dry matter. Formic acid increased weight gain per ton by 58% for direct-cut silage and 34% for wilted silage. The formic acid-formaldehyde mixture increased weight gain per ton by 41%, and formaldehyde increased it 68% (111).

Chemical composition. Formic acid, the formic acid-formaldehyde mixture, and formaldehyde decreased pH, ammonical nitrogen, acetic, butyric, and total acids. All three treatments increased residual sugar and insoluble nitrogen. Formic acid did not decrease lactic acid in direct-cut forage, but decreased its concentration in

wilted silage. The formic acid - formaldehyde mixture did not decrease lactic acid in direct-cut silage. Formaldehyde lowered lactate in direct-cut silage (111). Insoluble nitrogen is the amount of undegraded protein left in the silage after fermentation (111). Formaldehyde makes protein more insoluble by denaturing the protein; therefore, this protein may be more efficiently utilized by ruminants (111).

Formic acid addition to blight-damaged corn silage or excessively dried corn silage proved beneficial for all the experiments reviewed by Waldo (111). Formic acid or formic acid-propionic acid mixtures prevented deterioration of wet brewers' grains stored in laboratory silos and uncovered piles (111).

Comparisons of organic acids. Comparisons of organic acids are based on their ability to lower the pH to four. Titration experiments with fresh alfalfa showed that mineral acids were best, lactic and formic intermediate, and acetic and butyric were poorest for lowering pH when compared on an equivalent basis (58). Yahara and Nishibe (124) titrated direct-cut alfalfa and ranked the organic acids on their ability to lower pH: formic > lactic > acetic > propionic.

Aids to Silage Fermentation

Introduction. Aids to fermentation are those products that supply lactic acid-producing micro-organisms, nutrients required by lactic acid-producing micro-organisms, and enzymes and/or microbes that increase availability of carbohydrates and other nutrients

required by lactic acid-producing micro-organisms (13).

The need for fermentation aids has existed as long as silage making. Many forages do not have the proper amount of water-soluble carbohydrates to assure lactic acid fermentation.

Microbial cultures. As early as 1900, French researchers applied lactobacillus cultures to beet pulp silage, lowering its butyric acid content and producing a pleasant aroma. Watson and Nash (114) found effects of microbial cultures quite variable. There are many variables associated with producing an acceptable silage, such as the types and numbers of bacteria present on the crop, the type of culture used, the fermentable carbohydrate availability, and the moisture level of the silage (13).

Recent studies have shown favorable results of lactobacillus additive in silage (3, 57, 61), however, there are negative reports (114). Kirov (57) showed that a lactobacillus culture addition lowered silage pH and raised lactic acid values in vetch and clover silages. In the same year he reported good results with ensiled alfalfa (25 to 30% DM) treated with a 0.5% lactobacillus culture plus 1 to 1.5% molasses (57). Wieringa and Hengeveld (119) showed successful ensiling with a liquid culture of lactobacillus. McDonald et al. (73) reported less protein loss of silage treated with lactobacillus than in untreated silage, but dry matter losses and digestibilities were the same. A dried culture of lactic acid bacteria (1.0 kg per ton of fresh grass) increased fermentation rate, but depressed digestibility of dry matter in

Holsteins (33).

Influence of aids to fermentation upon nutrient preservation and dairy cattle performance. Bolsen (13) cited several researchers who worked with culture additives. Lactobacillus and Acetobacter oryzea cultures added to alfalfa to be ensiled lowered pH and peak temperature (33 vs. 50°C). There was no difference in milk production of cows fed the treated and untreated haylage. The same culture was added to direct-cut alfalfa stored in above-ground stocks. A. oryzea preserved more dry matter and protein than the control. Milk yield was similar, but milk fat level was higher for cows fed the treated haylage as well as milk produced per kg of feed (13). Corn silage (30% DM) treated with a fermentation controlling compound (mineral ingredients) caused slightly less dry matter consumption and fat-corrected milk production than the untreated corn silage (13).

Dry matter preservation, digestibility, and beef cattle performance related to aids to fermentation. A summary of the research cited by Bolsen (13), reveals that most of the researchers treated alfalfa with cultures of lactobacillus, A. oryzea, and Bacillus subtilis. In general, dry matter and protein preservation was either similar or slightly improved for treated silages as compared to untreated. Steers fed these treated haylages gained slightly faster with improved feed efficiency (13).

In experiments cited or performed by Bolsen (13), corn silage was treated with the same cultures as in the alfalfa trials.

Dry matter preservation for treated corn silage was usually better than for the untreated silage. Steers fed the culture treated silages generally gained faster with improved feed efficiency (13).

To summarize, Bolsen's (13) review on aids to fermentation indicate that variable success has been obtained in experiments. However, relatively few experiments have shown negative results. The greatest advantage of microbial additives may be their addition to ensiled alfalfa but the economic return is questionable (56).

Preservatives in Silage Production

Wilted haylage is desirable because it limits fermentation, reduces seepage from the silo, and increases consumption by cattle as compared to direct-cut silage (62). However, wilting is hampered by adverse weather conditions making it difficult to obtain an optimum dry matter in forage.

Certain direct-cut hay crop silages contain low levels of water soluble carbohydrates. Clostridial type organisms use protein as an energy source to produce undesirable fermentation products. Excessive wilting of haylage will cause heat-damaged non-degradable protein (62).

There are several kinds of silage preservatives as reviewed by Lusk (62). They are antibiotics, sterilants, and fatty acids.

Antibiotics

Zinc bacitracin. Dexter (28) treated full bloom alfalfa with 2, 10, and 50 ppm of five antibiotics individually and in mixtures. Initiation of fermentation was delayed only by the

zinc bacitracin at all levels. In this first experiment silage was ensiled in 946 ml jars, but in a later experiment with bunker silos, Dexter could not repeat the results of his first trial (62).

Rusoff et al. (94) ensiled direct-cut white Dutch clover treated with 5, 10, and 15 g of zinc bacitracin per ton and stored it in miniature silos. He compared this silage with molasses treated, sodium metabisulfite treated, and untreated forage. All treated forage had good aroma; however, steers consumed twice as much of the zinc bacitracin-treated silage. A further study in the same year with larger silos showed no difference in milk production of cows fed treated and controlled silages. Lactating cows required less zinc bacitracin-treated silage per unit of milk produced (95). Levels of butyric acids and pH were lower and levels of lactic, acetic, and propionic acids were higher in zinc bacitracin-treated haylage (93).

Alexander et al. (1) noted increased digestibility by sheep fed silage treated with zinc bacitracin. They concluded that zinc bacitracin could be used as a preservative in forages harvested at early stages of maturity. Pratt and Conrad (88) found no significant differences between zinc bacitracin and control silages in dry matter consumption and milk production. They noted reduced top spoilage of silage in treated silage as compared to controlled silage.

Lusk (62) cited other researchers (36, 37, 65, 89, 90) who found that zinc bacitracin-treated silages, in general, were not

better than controlled silages. Their results did not coinside with Rusoff's results (93, 94, 95). Rusoff's suggestion (93) that zinc bacitracin inhibits or depresses putrefactive spore forming bacteria tends to conflict with Langston et al. (59) who showed that Clostridium sporogenes grew well on media containing zinc bacitracin as a silage preservative (62).

Other antibiotics. Zinc bacitracin-treated silage was of better quality than those silages treated with terramycin, neomycin, penicillin, and aureomycin (28). Becker et al. (11) found proteolytic activity in Gahi millets treated with zinc bacitracin, chlorotetracycline, oleandomycin, oxytetracycline, penicillin, and streptomycin. These treated silages had lower dry matter consumption by lactating cows than the fresh millet. Antibiotic activity did not appear in milk of cows fed the treated millet except oleandomycin. Emery et al. (31) noted that tylosin treated alfalfa silage had higher lactic acid levels and that heifers gained 20% more when fed the treated forages. Tylosin activity had ceased in the silage after 30 days of storage (62).

Sterilants as silage preservatives. Lusk classifies these products as additives that tend to retard or inhibit undesirable fermentations in silage (62).

Sodium chloride. The research cited by Lusk (62) showed no advantage in using sodium chloride as a silage preservative.

Sulfur dioxide. Sulfur dioxide has been a silage addi-

with since 1885 (62). Sulfur dioxide treated forage retained a majority of the carotene after five months while the control lost over 75% of the carotene (62). Sulfur dioxide has been effective in increasing reducing sugars and lowering bacterial activity as compared to a control (62). Sulfur dioxide was difficult to properly distribute in silage and was inferior to sodium metabisulfite treated silage (62).

Sodium metabisulfite. Sodium metabisulfite has replaced sulfur dioxide at half the cost, and with easier and safer handling at ensiling. There are many conflicting reports of sodium metabisulfite as an effective silage sterilant. When added at a rate of 0.4% to silage with a dry matter content below 21%, the preservative has given increased weight gains of lambs, dry matter, crude protein, crude fiber, and energy digestibilities. Sodium metabisulfite reduced dry matter losses and conserved more carotene than compared to untreated silages. Sodium metabisulfite reduced butyric acid and ammonical nitrogen production in treated silages (62).

Sodium nitrite and calcium formate. Sodium nitrite seemed to control bacterial fermentation, but not yeast activity (122). Sodium nitrite has been mixed with calcium formate at a ratio of 3:20, respectively, and has been sold commercially in the United States and England (62). Aroma and physical appearance of sodium nitrite/calcium formate treated silage was superior to zinc bacitracin treated silage (101). However, there were no palatability

differences among cattle fed the silages. Gordon et al. (46) showed that the sodium nitrite/calcium formate mixture (2.43 kg/metric ton) and sodium metabisulfite lowered pH, improved silage odor, and preserved more carotene in grass-clover silage when compared to untreated silage.

Other researchers found sodium nitrite/calcium formate treated silage to be inferior to mineral acid treated silage (114). Hardison et al. (49) saw no difference in milk production from cows fed sodium metabisulfite, sodium nitrite/calcium formate, and control alfalfa haylage ensiled at 20 to 24% dry matter.

Fatty acids and related compounds as preservatives. Woolford (123) used a semi-micro assay technique to grow a number of organisms in yeast extract broth to screen the straight chain fatty acids as potential silage additives. The fatty acids assayed were formic through lauric (C_1-C_{12}) at pH levels of 3, 4, 5, and 6. All of the fatty acids screened appeared to have potential as a silage preservative. The C_1 through C_7 acids were effective in slowing the growth of spore forming bacteria while the higher fatty acids were more general in their preservative action. At a pH of 4, formic, acetic, and propionic acids inhibited yeast and mold growth more than the longer chain fatty acids (123). Butyric, valeric, and caproic acids have unpleasant odors and have been associated with undesirable silage fermentations (123). Therefore, the before mentioned acids probably would not be used as silage preservatives. The longer chain fatty acids are generally more expensive than the

shorter chain fatty acids (62).

Acetic acid. Mann and McDonald (64) treated Italian ryegrass (23.2% DM) with 0.45% formic, acetic, propionic, and varying mixtures of each and ensiled it in 3 kg capacity polyvinyl chloride silos. All acids restricted fermentation, but acetic and propionic were less effective than the others. Acetic acid had a lower pH and had the lowest level of water-soluble carbohydrates indicating less restriction of fermentation than with the other acids. Goering and Gordon (39) found that an acetic/propionic mixture was less effective in controlling mold growth in chopped alfalfa (45% DM) as was propionic acid alone at all levels of treatment from 0 to 1% (62). It appears that propionic acid is more effective than acetic acid when added to high dry matter silage (40 to 60% DM).

Propionic acid. Propionic acid at 0.1 and 0.2% levels slowed yeast growth, and at 0.4%, inhibited yeast growth without reducing numbers of lactic acid producing bacteria under laboratory conditions (47). Propionic acid at levels of 0.5 to 0.6% was a reliable preservative for forage that was difficult to ensile (48). Woolford (123) reported that propionic acid inhibited mold growth and did not inhibit the growth of lactic acid producing bacteria. Goering and Gordon (39) inhibited mold growth in alfalfa treated with 0.6% propionic acid, and prevented mold growth at 0.8% and 1.0% levels for 85 days.

Extensive mold and shrinkage occurred in a grass-clover

silage (50 to 65% DM) treated with 1.5% propionic acid and stored in a snow-fence silo (48). Yu and Thomas (126) found that fungal growth was reduced in alfalfa (wilted to 50 to 60% DM) treated with 0.4 and 0.8% propionic acid and ensiled in concrete stave silos (3.6 x 6.1 m). They reported that top spoilage was reduced by the 0.8% propionic acid treatment. Thomas (102) reported mold reduction in low-moisture alfalfa. Thomas (102) saw an increase in dry matter recovery of alfalfa stored in open snow-fence silo when treated with 1% propionic acid.

Lusk (62) cited several authors who indicated reduced ensilage temperature of forage treated with propionic acid. Propionic acid retarded aerobic fermentation of the silage after removal from the silo at the time of feeding (23). Britt et al. (17) treated chopped corn silage (35% DM) with either propionic, formic, 60% propionic/40% formic acid mixture, or 80% propionic/20% acetic acid mixture at 0.5, 1.0, and 2.0% levels. Silages were ensiled into polyethylene bags that were air-evacuated after filling. Lactic acid fermentation was totally inhibited at 2% addition of all acids, but formic acid was more effective than propionic at 0.5 and 1.0% additions. Propionic acid was most effective in delaying heating, growth of fungi, and days until spoilage during refermentation of silage.

Cottyn et al. (22) reported a significant increase in dry matter and protein digestion of Italian ryegrass treated with 4.4 l per metric ton of propionic acid. Yu Yu and Thomas (126) reported

improved protein digestion of alfalfa haylage treated with propionic acid. Addition of propionate improved digestibility of haylage in the top portion of the silo (126).

Lactating cows consumed more total dry matter in one trial but not in another when they were fed propionate-treated haylage (99). Cottyn et al. (22) reported increased dry matter consumption with propionate treated forage. Two reports (102, 126) conflict with Cottyn's findings in that there was no difference in dry matter consumption between treated and control silages.

Propionic acid treated haylage had reduced acid detergent fiber, cell walls, lignin, and acid detergent insoluble nitrogen than the controls (126). This suggests that less fermentation of water soluble carbohydrates occurred in the treated haylage. Control silages had a higher amount of acid detergent insoluble nitrogen than propionate treated haylage (126). The formation of acid detergent insoluble nitrogen occurs in high dry matter silage that have experienced excessive heating. Excessive heating causes the protein and the carbohydrates to condense and then accumulate in the lignin fraction of acid detergent fiber (40, 42, 103, 106, 125). The extent of heating of the silage is positively correlated with acid detergent insoluble nitrogen expressed either as a percent of dry matter or as a percent of total nitrogen ($r = .72$ and .80, respectively) (127). Increased ensiling temperature also caused a reduction in protein digestibility of haylage fed sheep (126).

There was no difference in milk production, milk solids or

butterfat content from cows fed the treated or control silage (126). Stallings et al. (99) observed no difference in milk production, milk fat, or fat-corrected milk, with the exception of one trial where fat production was reduced in cows fed propionate treated haylage. In three trials using propionic acid treated corn silage (42 to 47% DM), increases in dry matter intake and milk yield from cows fed the treated silage were observed (54). They concluded that propionic acid treatment of high dry matter silage appeared profitable (54). However, Stallings et al. (99) stated that when good quality haylage is available, no benefit is obtained from propionate treatment.

Sodium propionate. Sodium propionate is not as effective as propionic acid in reducing mold growth in haylage (39). Reduced consumption occurred in Italian ryegrass treated with a commercial product sold in France that contains sodium propionate as the active ingredient (22).

Ammonium isobutyrate. Ammonium isobutyrate was equal to sodium propionate and inferior to propionate in preventing mold in alfalfa silage (39). However, ammonium isobutyrate lowered ensiling temperatures more than propionate acid (39, 126). Propionic acid and ammonium isobutyrate equally reduced fungal counts in alfalfa haylage and both increased protein digestion over the controls (126). Thomas (102) noted a reduction in acid detergent fiber insoluble nitrogen of alfalfa treated with 0.75 to 2.1% ammonium isobutyrate or propionic acid. Acid detergent fiber insoluble

nitrogen was directly correlated to rise in ensiling temperature. The two acids had no influence on dry matter intake by sheep. However, dry matter intake of the treated silages was higher than the controls for one of two milk trials. There was no difference in milk yield when cows were fed either the treated or control silages (126).

Formaldehyde. Interest in the use of low levels of formaldehyde was increased after Brown and Valentine (18) observed that formaldehyde treated alfalfa silage contained lower ammonical nitrogen and total organic acids. Formaldehyde was equally affective as formic acid, or mixtures of acetic, propionic, formic, and formaldehyde but more effective as a bacteriostatic than when acetic or propionic acids were used alone (64). Lusk (62) cited other reports that showed that formaldehyde administered at 0.6 to 4.4% of dry matter reduced ammonical nitrogen and total titratable acidity.

Dry matter consumption and protein digestion were depressed when alfalfa was treated with 3.2 to 6.4% formaldehyde (18). However, formaldehyde added at 0.9% of the weight of alfalfa increased digestibility of both protein and dry matter over controls (105). Formaldehyde protected more protein of perennial ryegrass from ruminal degradation than protein in a control silage (81% vs. 17%, respectively). There was a net increase in amino acids absorbed from the small intestine of sheep fed the treated ryegrass (9).

Paraformaldehyde. Paraformaldehyde treated silage

is comparable to formic acid treated silage in terms of heifer average daily gain, feed conversion, and silage pH, but is less expensive than formaldehyde (110, 112, 113).

Formaldehyde and formic acid mixtures. Waldo (110) in 1977, reported that formic acid costs $13.75, formaldehyde $3.00, and paraformaldehyde $2.20 per metric ton of dry matter. As of 1977, formaldehyde had not been approved by the Food and Drug Administration for silage additive in the United States (110). Excessive treatment of hay-crop silage with formaldehyde reduces intake and protein digestion of the forage (18, 105). Favorable results in silage preservation and voluntary intake have been reported by the addition of formic acid to formaldehyde as a silage preservative (5, 7, 9, 24, 105, 112). The levels of ammonical nitrogen, total titratable acidity, lactic, propionic, and butyric acids were significantly lower in the formaldehyde and formic-formaldehyde treated silages than in the controls. The pH was lower and wool growth higher for formic-formaldehyde treated alfalfa silage (105). Best results with the formic-formaldehyde mixture have occurred at a level of 0.9% of dry matter.

Other acids. A study with caproic acid and formalin treated silages showed fermentation was greatly reduced by caproic acid and nearly stopped by formalin (84). Caproic acid increased water soluble carbohydrates in the silage (84). Caproic acid and 6 N hydrochloric acid added at ensiling or at silo opening, prevented aerobic deterioration but allowed temperature to rise in the ensiled

mass (83).

Benzoic acid treated silage had more nitrogen-free extract, digestible protein, lactic acid, and acetic acid than control silage. Milk yield was increased but not milk fat percentage for cows fed benzoic acid treated corn silage (100).

Summary. Whenever hay-crop silages can be ensiled at 30 to 40% dry matter with recommended ensiling procedures, little improvement in animal performance can be shown with treated haylages. Antibiotics become inactivated and sodium metabisulfite becomes oxidized at the higher temperatures encountered with high dry matter forage. These preservatives are more effective in low dry matter silages.

Sterilants show little value as silage preservatives. Propionic acid reduces mold growth and temperatures of high dry matter silages. Formaldehyde at low levels of treatment in low dry matter silage (18 to 30%) appears to be an effective preservative. Over protection, with formaldehyde alone, of protein from rumen degradation is reduced when formaldehyde is mixed with formic acid (62).

MATERIALS AND METHODS

Trial 1

Ensiling

Alfalfa was matured to 1/10 bloom, chopped to 6.25 mm in length, wilted to 44% dry matter (DM), weighed, and ensiled into four concrete bunker silos. Silos were 3.7 m wide by 11.0 m long. Haylage was transported by wagons and then unloaded into an elevator placed over the silo. Haylage was packed with a rubber-tired tractor to exclude oxygen. A commercial preparation of 10% propionic acid (Kemin Industries)[1] was applied at the chopper at a rate of 0.2%. Two silos received propionic acid treated haylage while the other two received untreated haylage. Black polyethylene plastic (0.1 mm thick) was placed over two of the silos, one with propionic acid treated haylage and one with untreated haylage.

Nylon Bag Technique

Twelve nylon bags containing 350 g of fresh haylage were buried 0.50 and 1.48 m from the floor and 2.74, 5.17, and 7.62 m from the back wall of each silo. Double stranded wire, soldered at one end (thermocouple), was tied to six bags located on one side of each bunker silo. These bags represented critical areas of fermentation occurring in the silo.

Temperature Readings

Daily haylage temperatures were measured from 24 nylon bags

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[1] Kemin Industries, Des Moines, Iowa.

by way of the thermocouple wires and a portable potentiometer. Readings were recorded for the first 49 days of storage.

Feeding Trial

Sixteen Holstein heifer calves weighing 147 to 237 kg were blocked by weight and randomly assigned to treatments (bunker silos). Calves were weighed once at the beginning and once at the end of the 3 mo trial. Calves were group fed once daily with weighbacks of haylage. Calves had free access to water. Dry matter intake (DMI) and average daily gain (ADG) were measured.

Feed Sampling

Haylage samples for dry matter determination were taken weekly during the feeding trial. All haylage was weighed as it was taken from the silos. This measurement was used to estimate total dry matter recovery (DMR). The nylon bags were recovered and frozen until analyses were performed.

Statistical Analysis

Data for temperature, nylon bag contents, and feeding trial was analyzed using procedure GLM of the 1979 version of the Statistical Analysis System (6).

Model 1. The model used to analyze temperature was:

$$Y_{ijkl} = \text{Mean} + \text{Cover}_i + \text{Treatment}_j + \text{Week}_k + (\text{Treatment} \times \text{Coveri})_{ij} + \text{Treatment} \times \text{Week}_{jk} + \text{Cover} \times (\text{Week})_{ik} + \text{Treatment} \times \text{Cover} \times (\text{Week})_{ijk} + \text{Error}_{ijkl}$$

Where: Y = each temperature observation, and

Cover = the effects of covered and uncovered,

Treatment = the effects of treatment with and without propionic acid, and

Week = the weeks of storage during temperature recording.

Model 2. The model used for analyzing composition of haylage in nylon bags was:

$$\dot{Y}_{ijklm} = Mean + C_i + T_j + P_k + A_l + TXC_{ij} + TXP_{jk} + CXP_{ik} + TXA_{jl} + CXA_{il} + TXCXP_{ijk} + PXA_{kl} + TXCXA_{ijl} + TXPXA_{jkl} + CXPXA_{jkl} + TXCXPXA_{ijkl} + Error_{kjklm}$$

Where Y = each variable measured, and

C = the effects of covered and uncovered,

T = the effects of treating and untreating with propionic acid,

P = the longitudinal position at which nylon bags were placed in the bunker silo, and

A = the altitude (top or bottom) at which nylon bags were placed in the silo. Position in the silo represents length of storage. The front position equals 82, middle 124, and back 141 days in storage.

Trial 2

Ensiling and Sampling of Haylage

Loads of 30% dry matter, 1/10 bloom alfalfa were weighed and

weighed and stored in two bunker silos measuring 3.7 m wide by 11.0 m long. Haylage was chopped to 6.25 mm and packed into silos with a rubber-tired tractor to exclude oxygen. Aliquots of haylage were taken as it was unloaded from the wagon into the elevator. Samples were mixed by hand in 19 l pails before frozen and/or analyzed for dry matter. Silo 1 was covered with 0.1 mm thick black polyethylene plastic. The top layer (5.0 cm) of haylage in silo 2 was treated with 100% propionic acid $(OCCO)^2$. Acid was evenly applied at a rate of 5.5% with a hand-held spray gun connected by hose to a power take-off (PTO) driven pump and 208 l capacity tank.

Nylon Bag Technique

Nylon bags containing 320 to 500 g of wilted and chopped alfalfa were buried in the same manner reported for Trial 1.

Silage Temperature Measurements

Temperatures of the nylon bag contents were measured as in Trial 1. Temperatures were recorded for the first 51 days of storage.

Feeding Trial

Eight Holstein heifer calves weighing 216 to 244 kg were paired by weight and randomly assigned to treatments (silos). Calves were assigned to individual pens (1.2 m wide x 4.9 m long). Calves were weighed on 3 consecutive days at the beginning and at 1 mo intervals during a 3 mo long feeding trial. Calves were fed ad libitum

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[2]Olwein Chemical Company, Olwein, Iowa.

amounts of haylage with weigh-backs recorded daily. Calves had free access to fresh water, high-phosphorus, and trace-mineral lick blocks. Mineral consumption, dry matter intake, average daily gain, and feed to grain ratio were measured.

Rumen Fluid Sampling

Rumen fluid samples via stomach tube were taken once during each of the 3 day weighing periods. Sample bottles contained 0.5 ml of saturated mercuric chloride to inhibit further microbial fermentation.

Feed Sampling

Aliquots of haylage were taken weekly from each silo during the growth trial and analyzed for dry matter. All haylage in the bunker silos was weighed as it was taken out. Nylon bags were recovered and frozen as the haylage around them was fed.

Statistical Analysis

Data for temperature, composition of haylage in nylon bags, composition of pre-trial rumen fluid, and animal performance during the feeding trial was analyzed by the statistical procedure used in Trial 1.

Model 1. The model used to analyze temperature was:

$$Y_{ijkl} = \text{Mean} + \text{Treatment}_i + \text{Week}_j + \text{Altitude}_k + \text{Treatment x week}_{ij} + \text{Treatment x altitude} + \text{Week x altitude}_{jk} + \text{Treatment x Week x Altitude}_{ijk} + \text{Error}_{ijkl}$$

Where Y = each temperature observation, and

Treatment = the effects of two treatments,

Week = the weeks of storage during temperature recording, and

Altitude = depth at which nylon bags were placed in the bunker silo

Model 2. The model used to analyze composition of haylage in nylon bags was:

$$Y_{ijkl} = \text{Mean} + \text{Treatment}_i + \text{Position}_j + \text{Altitude}_k + \text{Treatment} \times \text{Position}_{ij} + \text{Treatment} \times \text{Altitude}_{ij} + \text{Position} \times \text{Altitude}_{jk} + \text{Treatment} \times \text{Position} \times \text{Altitude}_{ijk} + \text{Error}_{ijkl}$$

Where Y = each variable measured, and

Treatment = the effect of two treatments,

Position = the effect of three longitudinal position in the silo, and

Altitude = the effect of two altitudes.

Position represented length of storage where the front of the silo equals 86, middle 100, and the back 144 days.

Model 3. The model used to analyze the composition of pre-trial rumen fluid was:

$$Y_{ij} = \text{Mean} = \text{Treatment}_i + \text{Error}_{ij}$$

Where Y = each variable measured, and

Treatment = the effect of two treatments.

The variables measured were used as covariates in the feeding trial.

Model 4. The model used to analyze animal performance during

the feeding trial period was:

$$Y_{ijk} = \text{Mean} + \text{Treatment}_i + \text{Period}_j + \text{Treatment x Period}_{ij} + \text{Error}_{ijk}$$

Where Y = each variable measured, and

Treatment = the effect of two treatments, and

Period = the division of days on the experiment.

Period one was from day 0 to 29 of the experiment, period two from day 30 to 59, and period three from day 60 to 91.

Chemical Analyses (Trials 1 and 2)

Nylon bag content and green chop analysis. Nylon bag contents were weighed in order to measure dry matter recovery. Dry matter analysis (2) was conducted on 25 to 32 g of wet haylage. A portion of the wet haylage was air dried for 2 to 3 days, through a 2 mm screen, and stored in labeled bottles. The remaining haylage was analyzed for pH and/or frozen in sealed plastic bags for future analyses.

Analyses on wet alfalfa

Haylage pH. Nine g of wet haylage was immersed 30 min in 60 ml of distilled water before pH was measured on a Orion pH meter (Model 501).

Lactic acid. Thirty-two g of haylage and 268 ml of distilled water were mixed in a Waring blender. The contents were refrigerated 30 min, reblended, and refrigerated again. The homogenate was filtered through Whatman No. 1 filter paper with a Buchner funnel. A celite filtering aid was also used. The extract was then

deproteinized by addition of (0.66 N), 90 ml $BaCl_2$ (98.8 g $BaCl_2$ · 2 H_2O/ 1 H_2O), and 45 ml $ZnSO_4$ (225.0 g $ZnSO_4$ · $7H_2O$/ 1 H_2O) to 90 ml of the silage extract. This mixture was filtered through No. 42 Whatman filter paper. Lactic acid determination (50) was performed on 25 ml of the deproteinized filtrate.

Ammonical nitrogen. Fifty ml of deproteinized haylage filtrate was subjected to ammonical nitrogen determination (2).

Non-protein nitrogen (NPN). Non-protein nitrogen content was determined on 50 ml of the deproteinized filtrate using the Kjeldahl apparatus (2).

Total nitrogen. A total nitrogen analysis (2) was determined on 1.5 to 2.0 g of the wet forage. Samples were weighed on the No. 42 Whatman filter paper (9.0 cm) and added to Kjeldahl flasks.

Gas-liquid-chromatography (GLC) analysis. Thirty g of wet haylage and 100 ml of 6.25% meta-phosphoric acid were homogenized in a Waring blender. The homogenate was squeezed through two layers of cheese cloth into a beaker. The mixture was refrigerated 30 min then filtered through No. 42 Whatman filter paper and a celite filtering aid. The filtrate was centrifuged at 12,000 x G for 20 min and the superatant was frozen in sample bottles. One microliter samples were injected into a 1.8 m x 3.1 mm I. D. stainless steel column containing 20% neopentyl glycol succinate (NPGS) plus 2% phosphoric acid liquid supported on 60/80 mesh fire brick. Chromatograph operating conditions, as set by Baumgardt (8) were modified

as follows: Column temperature 150°C, injection temperature 200°C, flame-ionization detector 195°C, nitrogen flow rate 30 ml/min, air flow rate 300 ml/min, and hydrogen flow rate 30 ml/min. The chromatograph used was a Varian Aerograph series 1400. All fatty acid peaks were recorded on a Sargent-Welch recorder.

Analyses on air dried samples. Acid detergent fiber (ADF) and ADF insoluble nitrogen were determined by the method of Goering and Van Soest (41). Neutral detergent fiber by the procedure of Van Soest and Wine (107) was conducted on the haylage. Ether extract content of the haylage was conducted on 1.0 g samples using the AOAC method (2).

Rumen fluid analyses. Rumen fluid pH was determined shortly after sampling. The fluid was then strained through three layers of cheesecloth to remove large particles. A 10 ml aliquot was acidified with 2 ml of 25% meta-phosphoric acid and centrifuged at 3,000 x G for 10 min. The supernatant was analyzed for volatile fatty acids on the same column used for silage extract. An additional 10 ml aliquot was centrifuged 10 min at 3,000 x G. The supernatant was acidified with 0.5 ml of 0.1 N HCl and analyzed for rumen ammonia by the method of Chaney and Marbach (19). Forty-five ml of rumen fluid were deproteinized with additions of 45 ml NaOH, 45 ml $BaCl_2$, and 22.5 ml $ZnSO_4$ (same reagents used to deproteinize the haylage). The filtrate was analyzed for lactic acid (50).

RESULTS AND DISCUSSION

Trial 1

Temperature

Average weekly temperature ranged from 32.8°C to 35.9°C for the covered haylage, but increased from 39.2°C in week 1 to 50.3°C ($P<.01$) in week 7 for the uncovered haylage (Table 2). Propionic acid (.02% addition) tended to reduce average weekly haylage temperature. The effects of cover and propionic acid treatment upon average weekly haylage temperature are illustrated in Fugure 1. An interaction ($P<.01$) between two factors, propionic acid treatment and cover, was observed which means that the effect of one factor was masked by the other. Covering lowered haylage fermentation temperature for all weeks by 8.5 and 13.9°C in treated and untreated haylages, respectively. Addition of propionic acid lowered haylage temperature for all weeks by 2.3 and 7.6°C in covered and uncovered haylages, respectively. Both covering and propionic acid treating lowered storage temperatures of haylage, but covering was more effective in this respect.

Similar research has shown that covered alfalfa haylage had lower temperatures at various positions in silos during 5 wk of storage as compared to three other silages (76). Propionic acid (1% at ensiling and 0.5% at feeding) (4) reduced heating in corn silage. Propionic acid addition to high dry matter corn silage at ensiling lowered silage temperatures during fermentation and feeding (54). Propionic acid (0.5, 1.0, and 2.0% additions) was more effective

TABLE 2. Mean temperature (C) of alfalfa haylage stored in bunker silos.

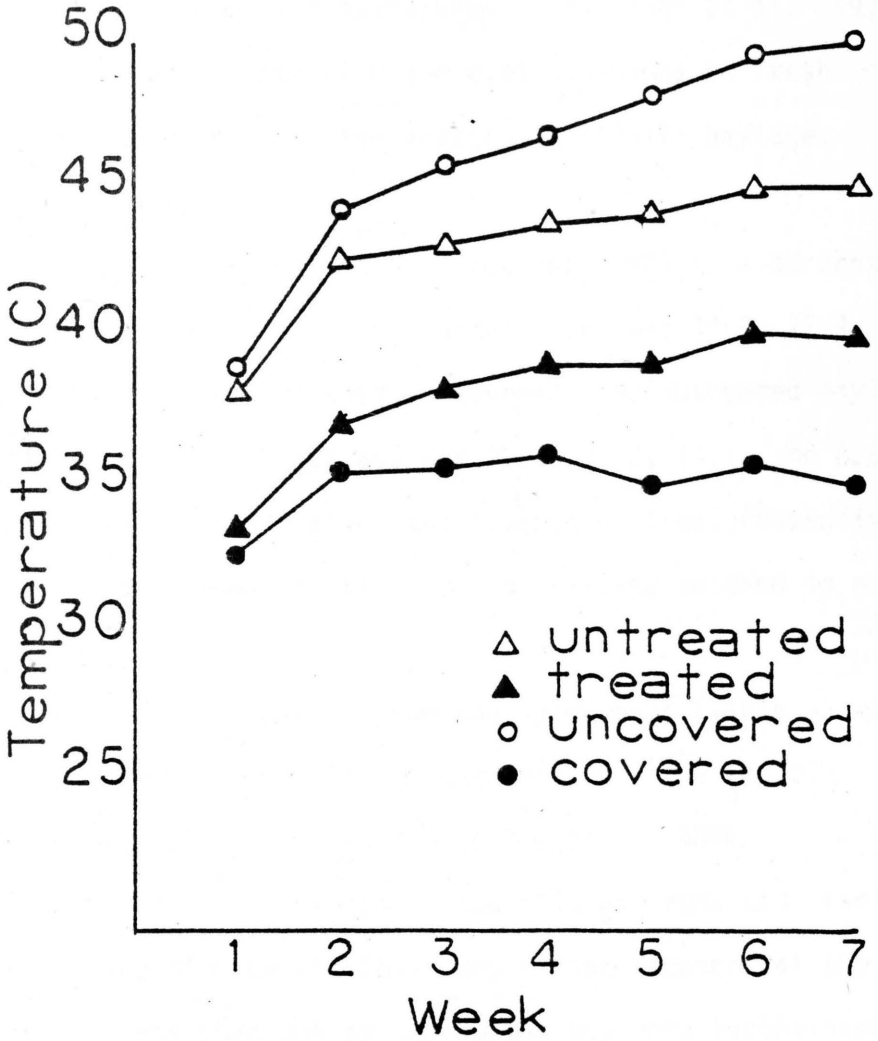
Variable[a]	Week**							$\bar{X}^b$
	1	2	3	4	5	6	7	
Covered**	32.8	35.6	35.8	36.2	35.2	35.9	35.2	35.2
Uncovered	39.2	44.5	46.0	47.1	48.4	49.8	50.3	46.5
Treated**	33.7	37.3	38.5	39.3	39.3	40.4	40.2	38.4
Untreated	42.8	43.4	44.0	44.4	45.3	45.3	45.3	43.3
$\bar{X}^b$	36.0	40.0	40.9	41.6	41.8	49.9	42.7	

[a]Error mean square: 62.63.

[b]Main effect means.

**Significance of interaction (P<.01).

Figure 1. The influence of covering and propionic acid on haylage fermentation temperature.


50
45
40
35
30
25
Temperature (C)
1
2
3
4
5
6
7
Week
△ untreated
▲ treated
o uncovered
● covered

than other acids in reducing heat during refermentation of corn silage that moved from air-tight barrels, at day 40 of storage, to open barrels (17). Propionic acid lowers fermentation temperature because it retards the growth of spore forming bacteria and mold (123) which cause higher temperatures. Stallings et al. (99), however, found propionate (1.0 and 0.5% additions to fresh forage) did not influence ensiling temperature of alfalfa haylage.

Dry Matter Recovery

The amount of dry matter recovered (DMR) as a percent of the total dry matter ensiled in the bunker silos was 73.9, 72.9, 57.3, and 56.3% for treated, covered, uncovered, and untreated haylage, respectively. Percent spoilage was 32.3, 27.0, 14.1, and 8.8% for uncovered, untreated, treated, and covered haylage, respectively. These values are based on the amount of haylage weighed in and out of the silos.

Dry matter recovered from the nylon bags (Table 3) was highest in covered and lowest in uncovered haylage ($P<.01$). Dry matter recovery of haylage at the bottom of the bunker silo was higher ($P<.01$) than at the top of the silo and remained nearly constant during storage ($P<.05$). Dry matter recovery at the top of the silo was less than DMR at the bottom and more inconsistent with storage time (Table 3).

Propionic acid increased DMR on the first and last periods of removal from the silo of nylon bags located at both top and bottom of the silo ($P<.05$). This increase in DMR, due to propionic acid addition, was more dramatic in uncovered haylage than in

TABLE 3. Dry matter recovery of alfalfa haylage in bunker silos as measured on nylon bag contents.

Variable[a]	Length of storage (days) 82[b]	124[c]	141[c]	$\bar{X}$
	%			
Covered**	86.0	91.8	92.7	90.2
Uncovered	66.2	87.0	78.2	77.1
Treated	73.4	88.8	93.4	85.2
Untreated	78.8	90.0	77.5	82.1
Top**	61.8	82.0	79.1	74.3
Bottom	90.3	96.8	91.8	93.0

[a]Error mean square: 77.81.

[b,c]Means with different superscripts are different ($P<.01$).

**Significance of interaction ($P<.01$).

covered haylage (P<.01).

Covering had negligable effects on dry matter recovery in treated haylage, but substantially increased DMR in untreated haylage (P<.01). Covering increased DMR by 24.7% in the top of the silo, but only slightly in the bottom (P<.05). There was a four-way interaction between treatment, position, cover, and altitude which indicated that cover and altitude accounted for the major differences in dry matter recovery. Propionic acid and length of storage (longitudinal position of nylon bags in the silo) had minor influences on DMR.

Spoilage of dry matter was the lowest in covered and the greatest in uncovered haylage. Dry matter recovery was highest in covered and lowest in uncovered haylage. Dry matter recovery was lower and more inconsistent with length of storage for haylage at the top of the silo than at the bottom.

Stallings et al. (99) reported that propionic acid increased dry matter recovery in the top of the silo, but not in the bottom. Dry matter recovery for covered haylage in the present experiment was similar and dry matter spoilage higher than values observed by Gordon et al. (44).

Haylage pH

Differences in haylage pH (Table 4) were most accentuated between values recorded for the top and bottom regions in the front section of the bunker silo (8.08 vs. 4.99) (P<.01). As storage time increased, haylage pH declined at the top (Table 4) and remained similar at the bottom of silos with uncovered haylage and

TABLE 4. The pH of haylage in nylon bags stored in bunker silos.

Variable[a]	Length of storage (days)			
	82[b]	124[b]	141[b]	$\bar{X}$
Covered**	5.76	4.92	4.83	5.17
Uncovered	7.31	6.47	6.24	6.67
Treated	7.00	5.90	5.14	6.01
Untreated	6.07	5.48	5.94	5.83
Top**	8.08	6.29	6.23	6.86
Bottom	4.99	5.10	4.85	4.98

[a]Error mean square: 13.24.

[b]Means were not different (P<.05).

**Significance of interaction (P<.01).

treated-covered haylage (P<.05). The pH of untreated-covered haylage at the top of the silo was lowest for the second period of silage removal. In the same haylage, pH at the bottom was similar for the first two periods of silage removal then decreased during the final period (P<.01).

Addition of propionic acid lowered haylage pH in the upper posterior section of the bunker silo and usually increased haylage pH in the front and middle regions of the silo (P<.01). Propionate was effective in lowering haylage pH in the front and middle sections of the silo that were covered, and in the back of silos with or without covering (P<.05). Propionic acid slightly decreased pH of haylage at the bottom of silos that were either covered or uncovered (P<.05). The addition of propionic acid dramatically decreased pH of covered haylage at the top of the silo, but to a lesser extent in uncovered haylage.

Covering reduced haylage pH more at the top of the silo than at the bottom (P<.05). Covering reduced the pH of haylage for all three periods of removal of nylon bags (P<.05).

Research with corn silage has shown that 1% addition of propionic acid lowered silage pH while the pH in aerobic deteriorated silage remained higher (4). McGuffey and Owens (76) reported that covering lowered haylage pH and that pH at the top of the bunker silo declined with increasing storage time. They also observed that pH in the bottom of the silo remained similar for all four periods of removal of nylon bags.

Lactic Acid

Differences in lactic acid content of the haylage (Table 5) were the greatest between the top and bottom of the silo ($P<.01$) and least between treated and untreated haylage. Lactic acid in the haylage was generally lowest for the first period of haylage removal and similar for the last two removals of the haylage as measured with nylon bags ($P<.05$). Lactate content of the haylage varied with length of storage the most in haylage stored at the top of the bunker silo ($P<.01$).

As length of storage time increased, lactic acid production increased in covered silos ($P<.01$). This effect was seen only in the top half of the bunker silos ($P<.01$). Covering increased lactate fermentation (Table 5) indicating that covering allowed a more efficient preservation of haylage than uncovering. A significant ($P<.05$) interaction between propionic acid treatment, cover, and length of storage has shown that propionic acid decreased lactate content of uncovered haylage only on the second period of nylon bag removal.

Lactate values in haylage with (2.96 % DM) or without covering (3.11% DM) were observed by McGuffey and Owens (76). Britt et al. (17) found that lactate levels were reduced in silage that had a 1% propionic acid addition.

Volatile Fatty Acids

Variations in the concentration of total volatile fatty acids (VFA) in the haylage were, in part, due to propionic acid

TABLE 5. Lactic acid of haylage in nylon bags stored in bunker silos.

Variable[a]	Length of storage (days) 82[b]	124[c]	141[c]	$\bar{X}$
	(% of DM)			
Covered**	2.36	3.34	2.96	2.89
Uncovered	1.65	2.32	2.03	2.00
Treated	2.07	2.71	2.61	2.46
Untreated	1.94	2.96	2.38	2.43
Top**	1.01	2.04	1.89	1.64
Bottom	3.00	3.63	3.10	3.24

[a]Error mean square: 0.72.

[b,c]Means with different superscripts are different ($P<.05$).

**Significance of interaction ($P<.01$).

treatment, covering, length of storage, and depth of haylage. Propionic acid, however, had no significant effect on the acetic acid concentration. Estimates of least square means were not available for covered, treated, and untreated haylage due to missing data. Effects of covering and propionic acid upon volatile fatty acid content of haylage could not be evaluated.

Acetate was the major volatile fatty acid produced in all haylages. Both acetic acid and total volatile fatty acid concentrations declined with increased storage time ($P<.01$) Table 6). All interactions between treatment, covering, length of storage, and altitude were significant ($P<.01$) for both acetate and total VFA.

Britt et al. (17) and Stallings et al. (99) noted that a 1% propionic acid addition lowered the acetate content of corn silage and alfalfa haylage.

Nitrogen Fractions

Total nitrogen content of haylage was not significantly different between treated or untreated haylage or covered and uncovered haylage. In addition, depth of haylage and storage time had no effect on total nitrogen content of the haylage (Table 7). Stallings et al. (99) noted that total nitrogen was slightly higher in propionic acid treated haylage.

Non-protein nitrogen (NPN) content of the haylage (Table 7) was lower at 82 days of storage than at 141 days ($P<.01$). Non-protein nitrogen was higher in haylage stored in the bottom half of

TABLE 6. Volatile fatty acids in alfalfa haylage stored in bunker silos.

Acid	Length of storage (days) 82	124	141	SE[f]
	(mM/100 g DM)			
Acetic	11.06[a]	11.05[a]	9.69[b]	0.26
Total	13.89[c]	12.29[d]	10.41[e]	0.28

[a,b]Means with different superscripts are different ($P<.05$).

[c,d,e]Means with different superscripts are different ($P<.01$).

[f]Standard error of the means.

TABLE 7. Total nitrogen and nitrogen fractions of alfalfa haylage in nylon bags placed in bunker silos.

Main effect	Total nitrogen	Non-protein nitrogen	Acid detergent fiber insoluble nitrogen	Ammonical nitrogen	
	(% DM)				– (% TN[a]) –
Covered	3.33	0.68	0.94**	0.22	6.73
Uncovered	3.46	0.68	1.29	0.28	7.81
Treated	3.40	0.75**	1.15	0.27	7.68
Untreated	3.39	0.62	1.08	0.24	6.84
Length of storage					
82 days	3.31	0.58[b]	1.41[b]	0.24	7.11
124 days	3.54	0.68[b,c]	1.02[c]	0.25	6.94
141 days	3.34	0.78[c]	0.91[c]	0.27	7.76
Altitude					
Top	3.37	0.58**	1.42**	0.25	7.43
Bottom	3.42	0.78	0.81	0.25	7.11
MSE[d]	0.13	0.03	0.11	0.02	11.14

[a]Total nitrogen.

[b,c]Group means with different verticle superscripts are different (P<.01).

[d]Error mean square.

**Significance (P<.01).

the silo (P<.01) and in haylage treated with 0.02% propionic acid (P<.01) (Table 7). Non-protein nitrogen content was higher in propionate treated/uncovered haylage than in untreated/uncovered haylage, but was similar between treated or untreated covered haylage (P<.01). Covering reduced NPN in the propionic acid treated haylage (P<.01). Covering slightly reduced non-protein nitrogen content of haylage in the middle of the bunker silo (P<.01). Haylage in the upper anterior and upper middle regions of the covered bunker silos was higher in NPN than haylage of the same areas in uncovered silos (P<.01). The later results are contrary to that of McGuffey and Owens (76). They reported that covering reduced non-protein nitrogen. They noted, however, that NPN was higher at the bottom of the silo.

Ammonical nitrogen, presented in Table 7 as percent of the dry matter or as percent of the total nitrogen, was similar in concentration regardless of propionic acid addition, depth of haylage, or length of storage. Covering, however, tended to lower ammonical nitrogen (P<.01). Advancing storage time tended to increase ammonical nitrogen of haylage at the bottom of covered silos. Length of storage had no effect on ammonical nitrogen in haylage at the top of the silo. Other investigators indicated that ammonical nitrogen was higher in haylage stored at the bottom of bunker silos (76). A four-way interaction between treatment, covering, length of storage, and altitude (P<.01), could not be explained biologically.

Acid detergent fiber insoluble nitrogen (ADFIN) (Table 7)

was lowest in haylage at the bottom of the bunker silo and highest at the top ($P<.01$). Acid detergent fiber insoluble nitrogen was lower in haylage at the lower level of the silo because of the lower fermentation temperature. Although the effect of depth of haylage upon fermentation temperature was not analyzed, it was highly speculated that ensiling temperature was higher at the top of the silo. Ensiling temperature has been highly correlated to ADFIN (106, 127). As storage time progressed, ADFIN content declined ($P<.01$) in haylage at both top and bottom of the silo. This decrease occurred because of more anaerobic conditions in haylage at the middle and posterior sections of the bunker silo. Anaerobic conditions are associated with lower fermentation temperature (71). Acid detergent fiber insoluble nitrogen content in haylage representing the longest storage time was higher at the bottom of the silo than at the top for no apparent reason. Covering reduced ADFIN in haylage stored at either the top or bottom of the bunker silo ($P<.01$) due to a more anaerobic environment. Other researchers have reported higher levels of ADFIN in haylage that was uncovered or at the top of the silo (76). Stallings et al. (99) did not reduce ADFIN with addition of propionic acid.

Acid Detergent Fiber

Length of storage beyond 82 days did not change acid detergent fiber (ADF) content in haylage (Table 8). Acid detergent fiber content in haylage increased from bottom to top of the silo ($P<.01$) (Table 8) and was higher in haylage not treated with propionic acid

TABLE 8. Acid detergent fiber in alfalfa haylage stored in bunker silos.

Variable[a]	Length of storage (days) 82	124	141	$\bar{X}$
	(% of DM)			
Covered**	40.4	36.7	38.3	38.5
Uncovered	48.4	46.7	45.2	46.8
Treated*	42.4	40.5	39.3	40.8
Untreated	46.5	42.9	44.2	44.5
Top**	48.2	46.5	44.1	46.3
Bottom	40.7	36.9	39.4	39.0

[a]Error mean square: 25.42.

*Significance ($P<.05$).

**Significance ($P<.01$).

(P<.05). Other investigators showed that ADF was 37.6 vs. 39.1 (% DM) for propionate treated and untreated haylage, respectively (99).

Covering the haylage reduced ADF by 13.2% and 3.4% in haylage stored at the upper and lower levels of the silo, respectively (P<.01).

Chemical composition of haylage varied considerably from top to bottom of the silo and between covered and uncovered haylage. Additions of propionic acid had little effect on improving silage quality as based on chemical composition. Like propionic acid, length of storage had a minor influence upon changing chemical composition in haylage. Generally, the front of the silo had lower quality haylage than either the middle or back sections of the bunker silo.

In this experiment, heifer growth rate was lower than NRC (79) standards. However, average daily dry matter and nitrogen intakes were more than adequate to support gains achieved in this trial. The data indicates that haylage preserved by both covering and propionic acid treating was inadequate in energy to support the growth of young dairy heifers.

Heifers fed covered or untreated haylage gained faster than those fed either uncovered or treated haylage. All haylage was inadequate in energy to support growth of replacement heifers.

Animal Performance

Group dry matter intakes (kg/day) were 6.71, 6.51, 6.30,

TABLE 9. Average daily gain of heifer calves fed alfalfa haylage.

Haylage type	Average daily gain	SE	P>F
	(kg)		
Covered	0.61	0.01	0.01
Uncovered	0.54	0.01	0.01
Treated	0.54	0.01	0.01
Untreated	0.60	0.01	0.01

and 6.91 for heifers fed covered, uncovered, treated, and untreated haylage, respectively. Apparent feed to gain ratios (kg feed/kg gain) calculated from group averages were 11.10, 12.14, 11.73, and 11.51 for covered, uncovered, treated, and untreated haylage, respectively. Cottyn et al. (22) reported increased dry matter intake of propionate treated haylage while Yu Yu and Thomas (126) and Thomas (102) found no differences in dry matter intake between treated and control silages. Calves fed covered or untreated haylage gained faster than calves fed uncovered or treated haylage ($P<.01$) (Table 9). The covered haylage had a higher recovery energy as estimated by dry matter recovery (71) than any other haylage. The covered haylage also had more available protein (total nitrogen - ADFIN % DM) (6.25) for bacterial protein synthesis. The higher energy recovery and available protein could support a faster growth in heifers. No explanation could be given for the growth rate observed in heifers that consumed the untreated haylage.

Trial 2

Composition of Pre-ensiled Haylage

Chemical composition of pre-ensiled haylage was nearly identical for both silos. Composition of alfalfa haylage ensiled is presented in Table 10. The dry matter (DM) content of haylage going into the silos was higher at the middle of the silo ($P<.05$) due to wilting prior to ensiling. Total nitrogen and lactic acid were lower in haylage ($P<.01$) stored at the front of the silo. Areas of grass were in the field of alfalfa haylage that was ensiled in the

TABLE 10. Composition of alfalfa before ensiling.

Variable	Means		SE[a]	P>F
	Control	Propionate treated		
Dry matter (%)	31.64	32.69	0.72	NS
pH	5.77	5.76	0.02	NS
	—— (% of DM) ——			
Lactate	0.61	0.67	0.08	NS
Total nitrogen	3.23	3.02	0.11	NS
Non-protein nitrogen	0.24	0.24	0.02	NS
Ammonical nitrogen	0.04	0.03	0.01	NS
Acid detergent fiber insoluble nitrogen	0.37	0.34	0.12	NS
Cell solubles[b]	52.96	55.57	1.23	NS
Neutral detergent fiber	47.04	44.43	1.23	NS
Acid detergent fiber	34.71	33.10	0.64	NS
Hemicellulose[c]	12.33	11.58	1.40	NS
Ether extract	1.91	1.99	0.05	NS

[a]Standard error of the means.

[b]Cell solubles = 100-NDF.

[c]Hemicellulose = NDF-ADF.

front of the bunker silo. Grasses are lower in protein (79) and organic acids (32) as compared to alfalfa. Lactic acid was not mentioned in the literature as being a normal constituent of fresh forage (30). Fermentation of organic acids in the fresh haylage to lactate (30, 53, 87, 115) may have occurred while the haylage was in route from the field to the silo. Alfalfa in the front of the silo was higher in acid detergent fiber ($P<.01$). A statistical interaction between treatment and position in the silo ($P<.05$) was observed for acid detergent fiber and ether extract but differences among values were minor. The interaction occurred because variation among loads of alfalfa haylage occurred due to the grass content. Grasses such as timothy and orchard-grass are typically higher in acid detergent fiber than alfalfa (79).

Total nitrogen values of the pre-ensiled alfalfa are similar to the values obtained by Goering et al. (42). Acid detergent fiber insoluble nitrogen values were higher than those observed by other researchers (42). Ammonical and non-protein nitrogen values were similar to those reported in the literature (30). Acid detergent fiber values were similar, but neutral detergent fiber and hemicellulose values were slightly lower than those observed by Goering et al. (42).

Temperature

Average weekly haylage temperature ranged from 32.5 to 35.4^{o}C in the control silage (covered), but increased from 34.0^{o}C in week 1 to 48.3^{o}C in week 6 in the propionic acid topically

treated haylage (P<.01) (Table 11). In the control haylage, mean temperatures from bottom to top of the bunker silo ranged from 31.9 to 35.4°C. In the treated haylage, however, temperatures from bottom to top ranged from 36.8 to 49.2°C (P<.01). The haylage in the top of the silo had more aerobic fermentation causing higher temperatures (71). The statistical interactions of treatment by week and treatment by week by altitude are graphically illustrated in Figures 2 and 3. In Figure 2, weekly temperature rose steadily for the treated haylage while temperature of the control haylage remained steady throughout storage. In Figure 3, temperatures of haylage at the base of the bunker silos remained nearly constant during storage, especially in the control silo. Haylage in the upper level of the treated silo was severely heated.

Propionic acid did not lower temperatures in this trial. This is probably due to 17.5 cm of rainfall that occurred on June 25, 1980, which diluted the concentration of propionic acid. Stallings et al. (99) noted that propionic acid did not lower haylage temperature in one experiment. In the present trial, rising temperature of haylage during the first 7 wk of storage was uncontrolled by topical addition of propionate.

Dry Matter Recovery and pH

Treatment of haylage with propionic acid, length of storage and depth of haylage had no influence upon dry matter recovery and haylage pH. Dry matter of nylon bag contents was higher for the control haylage (P<.05) and dry matter recovery tended to be higher

TABLE 11. Mean temperature (C) of alfalfa haylage stored in bunker silos.

Treatment**	Week**							$\bar{X}^a$	SE[b]
	1	2	3	4	5	6	7		
Acid treated	34.0	39.4	39.3	44.5	47.0	48.3	48.3	43.0	0.61
Control	32.5	35.4	33.5	33.5	34.8	33.5	32.6	33.7	0.56
$\bar{X}^a$	33.3	37.4	36.4	39.0	40.9	40.9	40.4		

[a]Main effect means.

[b]Standard error of mean for treatment, not week.

**Significance of interaction (P<.01).

Figure 2. Temperature of control and treated haylage during storage.

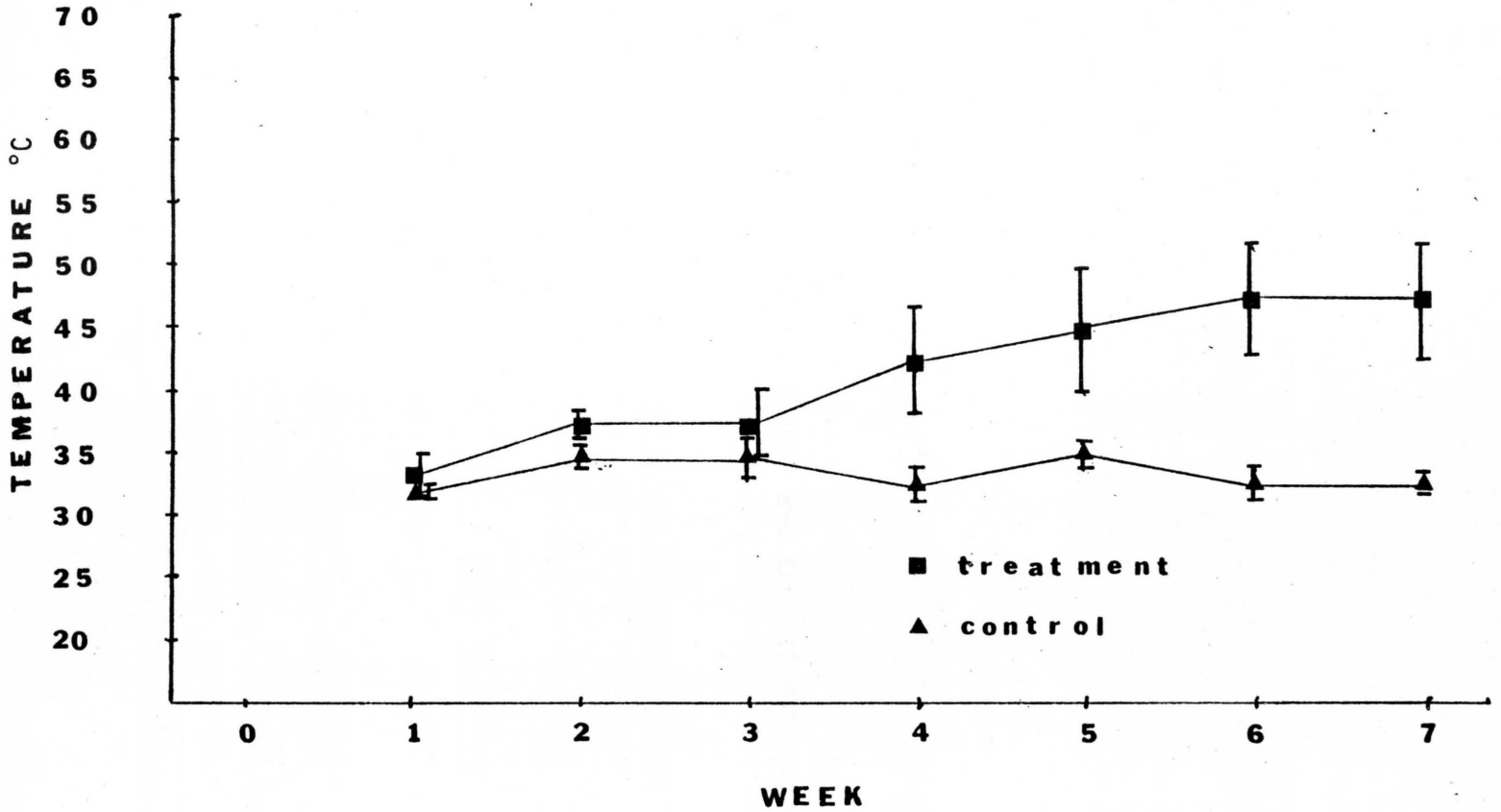
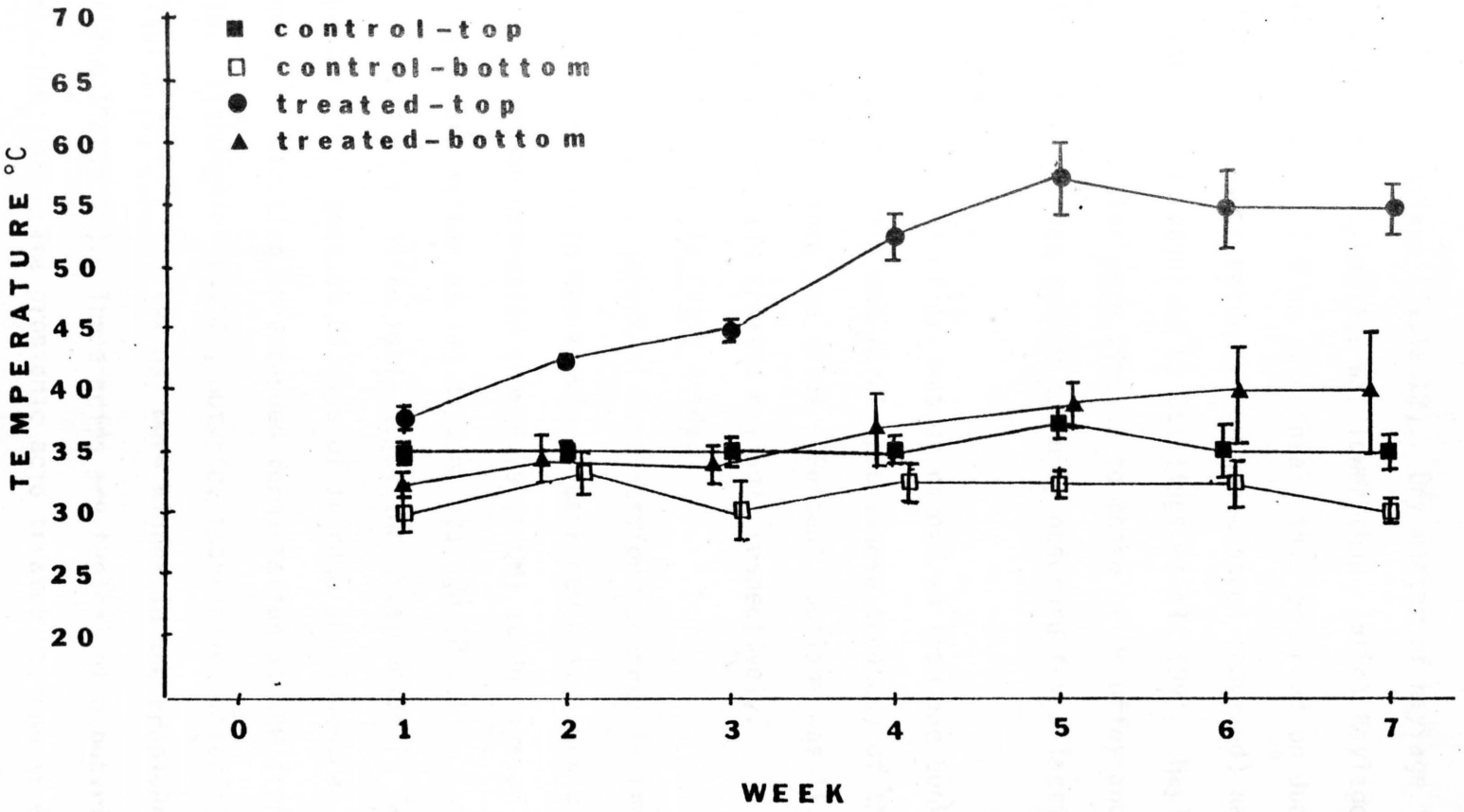

TEMPERATURE °C
70
65
60
55
50
45
40
35
30
25
20
0
1
2
3
4
5
6
7
WEEK
treatment
control

Figure 3. The influence of treatment and depth of haylage on ensiling temperature.


control-top
control-bottom
treated-top
treated-bottom
TEMPERATURE °C
70
65
60
55
50
45
40
35
30
25
20
0
1
2
3
4
5
6
7
WEEK

for control haylage (Table 12). Dry matter of haylage in the uncovered (treated) haylage was lower than control haylage dry matter; probably due to 17.5 cm of rainfall that occurred on June 25, 1980. Haylage dry matter recovery for the control (covered) haylage was similar to that observed by Stallings et al. (99). Haylage pH for the control bunker were similar to those of McGuffey and Owens (76), but are higher than the pH commonly observed for a lactic acid fermentation (16).

The amount of dry matter recovered from the bunker silos was 62.7% (control) and 42.2% (propionate treated) of the original dry matter put into the silos. Percent spoilage was 11.0 and 8.9 for the control and treated haylage, respectively.

Lactic and Volatile Fatty Acids

A greater amount of fermentation occurred in the front region of the silo than in the middle or back regions. Lactic acid decreased in concentration from 6.79 (% DM) at 86 days of storage to 3.55% of dry matter at 144 days of storage ($P<.01$). Concentrations of individual volatile fatty acids and total volatile fatty acids tended to be higher at 86 days of storage and lowest at 144 days of storage, indicating an extended fermentation at the front of the silo. Isobutyric ($P<.01$), butyric, isovaleric, valeric, and total volatile fatty acids ($P<.05$) were higher in the propionate-treated haylage (Table 12). These acids are typical of a butyric acid fermentation (30). The propionic acid treated haylage is characteristic of clostridial or butyric acid fermentation (30). The dry

TABLE 12. Dry matter, dry matter recovery, and pH of alfalfa haylage after storage.

Parameter	Means		SE[a]	P>F
	Control	Propionate		
Dry matter (%)	29.7	26.0	1.21	0.05
Dry matter recovery (%)	86.3	77.6	4.91	NS[b]
pH	5.27	6.01	0.35	NS

[a]Standard error of parameter means.

[b]Non-significant difference.

matter content of the control haylage was high enough above the critical dry matter content (28%) that a clostridial fermentation would not probably occur. Concentrations of acids (Table 13) for both haylages are above those levels recommended for high quality haylage (16). The control haylage is considered slightly inferior, whereas, the propionic acid treated haylage is thought to be grossly inferior in quality as indicated by animal performance and chemical analysis of the feedstuff.

Volatile fatty acid analysis for both haylages closely resembles that of McGuffey and Owens (76) who compared haylage ensiled at 34 or 43% dry matter. Several investigators have reported reduced haylage fermentation as dry matter of the ensiled material increased (40, 41).

Nitrogen Fractions

Ammonical nitrogen was higher in the propionic acid treated (0.68% DM) than in the control (.31% DM) haylage (Table 14). The increased level of ammonical nitrogen in the propionate treated haylage is typical of low dry matter silage (40, 41). Levels of ammonical nitrogen recorded for this experiment agree with values reported in the literature (76). Ammonical nitrogen tended to be higher in the front of both bunker silos, although not significantly.

Acid detergent fiber insoluble nitrogen appeared to be higher in top and front of the silo. Acid detergent fiber insoluble nitrogen values were highly correlated to haylage temperature which was higher in the top region of both silos and especially higher in

TABLE 13. Lactic and volatile fatty acids of alfalfa haylage stored in bunker silos.

Acid	Means		SE[a]	P>F
	Control	Treatment		
	— (mM/100 g DM) —			
Lactic	4.85	5.25	3.30	NS
Acetic	50.27	34.78	5.61	NS
Propionic	2.80	4.96	1.45	NS
Isobutyric	0.15	2.99	0.69	0.01
Butyric	2.15	26.86	7.34	0.05
Isovaleric	0.26	5.62	1.36	0.05
Valeric	0.05	2.16	0.69	0.05
Total volatile fatty acids	55.68	77.37	14.30	0.05

[a]Standard error of the means.

TABLE 14. Total nitrogen and nitrogen fractions of alfalfa haylage stored in bunker silos.

Variable	Means		SE[a]	P>F
	Control	Propionate		
	—— (% of DM) ——			
Total nitrogen	3.38	3.24	0.25	NS
Non-protein nitrogen	1.32	1.34	0.19	NS
Ammonical nitrogen	0.31	0.68	0.09	0.05
Acid detergent fiber insoluble nitrogen	0.30	0.45	0.12	NS

[a]Standard error of the means.

the treated (uncovered) silo. This data agrees with that of McGuffey and Owens (76) and Yu Yu and Thomas (127). Acid detergent fiber insoluble nitrogen is an accurate estimate of heat-damaged protein and was positively correlated with heating either as a percent of dry matter or as a percent of total nitrogen (r = 0.72 and 0.80, respectively) (127). The extent of heating of the haylage during fermentation has been reported to be negatively correlated with digestibility of the dry matter, nitrogen, and nitrogen balance (r = -0.33, -0.81, and -0.49, respectively) (127). Van Soest (106) reported that ADFIN values of 7% (as percent of total nitrogen) are normally found in fermented forages. Forages with ADFIN values (% of total nitrogen) of 14% or above are considered to be heat damaged (40).

Plant Fiber Fractions, Cell Solubles, and Ether Extract

Differences in cell solubles, neutral detergent fiber ($P<.05$) and acid detergent fiber ($P<.01$) (Table 15) were observed between the two treatments used on haylage. Propionate treated haylage had a greater fermentation of cell solubles than the control; therefore, had higher neutral detergent fiber and acid detergent fiber values. The propionate treated haylage (uncovered) had a higher temperature recorded during fermentation. Other researchers (106, 127) have demonstrated a close relationship between the extent of heating and values for acid detergent fiber, lignin, and ADFIN.

A comparison of the chemical composition of haylage before

TABLE 15. Cell wall constituents, cell solubles, and ether extract of haylage stored in bunker silos.

Variable	Mean		SE[a]	P>F
	Control	Propionate		
	(% of DM)			
Cell solubles[b]	57.47	51.62	1.70	.05
Neutral detergent fiber (lignin, cellulose, hemicellulose) (NDF)	42.36	48.38	1.67	.05
Acid detergent fiber (ADF)	36.16	42.52	1.23	.01
Hemicellulose[c]	6.20	5.91	0.67	NS
Ether extract	4.74	5.48	0.43	NS

[a]Standard error of the means.

[b]100-NDF.

[c]NDF-ADF

and after storage (Tables 10 and 15) indicate that hemicellulose was the most unstable cell wall constituent of both haylages. There was a 51% reduction of hemicellulose in both haylages during fermentation. The uncovered haylage (treated), however, had a slight increase in neutral detergent fiber and a marked increase in acid detergent fiber while hemicellulose decreased substantially. Goering et al. (42) found the same trend in their study and noted also that hemicellulose was inversely related to temperature during fermentation.

Fermentation had little effect on ether extract values of the haylage. Levels of ether extract probably increased due to the loss of other dry matter constituents in the initial haylage.

The control (covered) haylage had less fermentation than the treated haylage (uncovered); however, both haylages experienced reduction in cell solubles, hemicellulose, and dry matter content. Both haylages experienced increases in non-protein nitrogen, ammonical, acid detergent fiber, and ether extract. These increases are typical of silage fermentation (42, 60).

Animal Performance

Rumen fluid composition of dairy heifers before assignment to treatments is presented in Table 16. Isobutyrate ($P<.05$) and ammonical nitrogen tended to be higher in the group of calves assigned the diet containing propionic acid treated haylage. One calf in the same group had consistently higher rumen fluid ammonia (26 mg/100 ml) values on the initial and second of four sampling

TABLE 16. Composition of rumen fluid of dairy heifers before assignment to experimental haylage.

Constituent	Means		SE[a]	P>F
	Control	Propionate		
pH	7.09	7.19	0.09	NS
Acetate (molar %)	66.51	67.29	0.79	NS
Propionate (molar %)	18.93	18.66	0.56	NS
Isobutyrate (molar %)	1.19	1.49	0.07	0.05
Butyrate (molar %)	8.22	7.54	0.55	NS
Isovalerate (molar %)	2.40	2.77	0.38	NS
Valerate (molar %)	2.83	2.25	0.22	NS
Lactate (g/100 ml)	0.03	0.03	0.002	NS
Ammonia (mg/100 ml)	9.48	16.38	2.96	NS

[a]Standard error between the means.

periods. Rumen ammonia values were measured as an indicator of differences in protein degradability. Heat-damaged protein has been reported to be less degraded to ammonia in the rumen as unheated protein (96).

Composition of rumen fluid samples during the feeding trial is presented in Table 17. Calves fed the propionic acid treated haylage had a 2.0 fold increase in rumen butyrate levels while those fed the control haylage had a 1.7 fold increase (Tables 16 and 17). A comparison between rumen fluid volatile fatty acids measured before and during the feeding trial indicates that acetate and propionate levels decreased by 10% and 3%, respectively, while butyrate isobutyrate, and isovalerate increased substantially. Volatile fatty acids in the haylage were not reflected in the rumen fluid except for butyric acid. Levels of butyric acid was ten times higher in the treated haylage than control haylage and was higher ($P<.01$) in the rumen fluid of those calves fed the propionic acid treated haylage. The control haylage had lower amounts of isobutyric, isovaleric, and valeric acids ($P<.01$); however, isobutyric ($P<.01$), isovalerate ($P<.05$), and valerate were higher in the rumen fluid of those calves fed the control haylage.

Rumen ammonia was not significantly different between calves fed the two haylages (Table 17). This indicates that there was no apparent difference in haylage protein degradability.

Rumen lactic and propionic acid contents were higher in the

TABLE 17. Composition of rumen fluid for heifers fed control and propionic acid treated haylage.

Constituent	Means		SE	P>F
	Control	Propionate		
pH	7.14	7.07	0.03	NS
Acetate (molar %)	59.85	59.64	0.38	NS
Propionate (molar %)	15.92	15.37	0.20	NS
Isobutyrate (molar %)	3.20	2.91	0.07	0.01
Butyrate (molar %)	14.20	15.67	0.29	0.01
Isovalerate (molar %)	4.29	3.86	0.14	0.05
Valerate (molar %)	2.91	2.70	0.13	NS
Lactate (g/100 ml)	0.04	0.03	0.002	0.05
Ammonia (mg/100 ml)	13.34	15.55	1.59	NS

first 29 days (period 1) and were similar between periods 2 and 3 ($P<.01$) of the feeding trial for both groups of calves. Acetic acid was lower ($P<.01$) and butyric acid higher ($P<.01$) in the rumen fluid of calves fed both haylages the second period (day 29 to day 59) of the feeding trial. Rumen fluid butyrate content of both groups of calves varied among all three sampling periods during the feeding trial ($P<.01$). A statistical interaction between treatment and period was observed for rumen acetate and propionate ($P<.01$) levels. This interaction indicated that there was a slight difference between treatments occurring within periods of the feeding trial.

Heifers gained slightly faster and consumed more of the control haylage ($P<.01$) with slightly better feed efficiency than those heifers fed the treated haylage (Table 18). Average daily gain for heifers fed the control haylage increased from the end of period 1 to the end of period 2 (Figure 4). Those calves fed the treated haylage started to gain faster in the third than in the previous periods. There was a corresponding increase in dry matter intake by heifers fed the control haylage, also, during the second period (Figure 5). This may be due to the increase in dry matter content of the control haylage. Dry matter intake throughout the entire trial was higher for calves fed the control haylage partly because dry matter content was higher. Gordon et al. (43) reported that dry matter consumption was linearly correlated to dry matter content ($r = 0.53$), especially for those haylages with less than 50% dry matter. The 12.8% difference in dry matter content between the

TABLE 18. Growth of dairy heifers fed control and propionic acid treated alfalfa haylage.

Parameter	Means		SE[a]	P>F
	Control	Propionate		
Average daily gain (kg)	0.58	0.44	0.05	NS
Dry matter intake (kg)	7.25	5.81	0.08	0.01
Feed/gain	12.85	19.28	3.90	NS

[a]Standard error between the means.

Figure 4. Weight gains of heifers fed control and treated haylage.

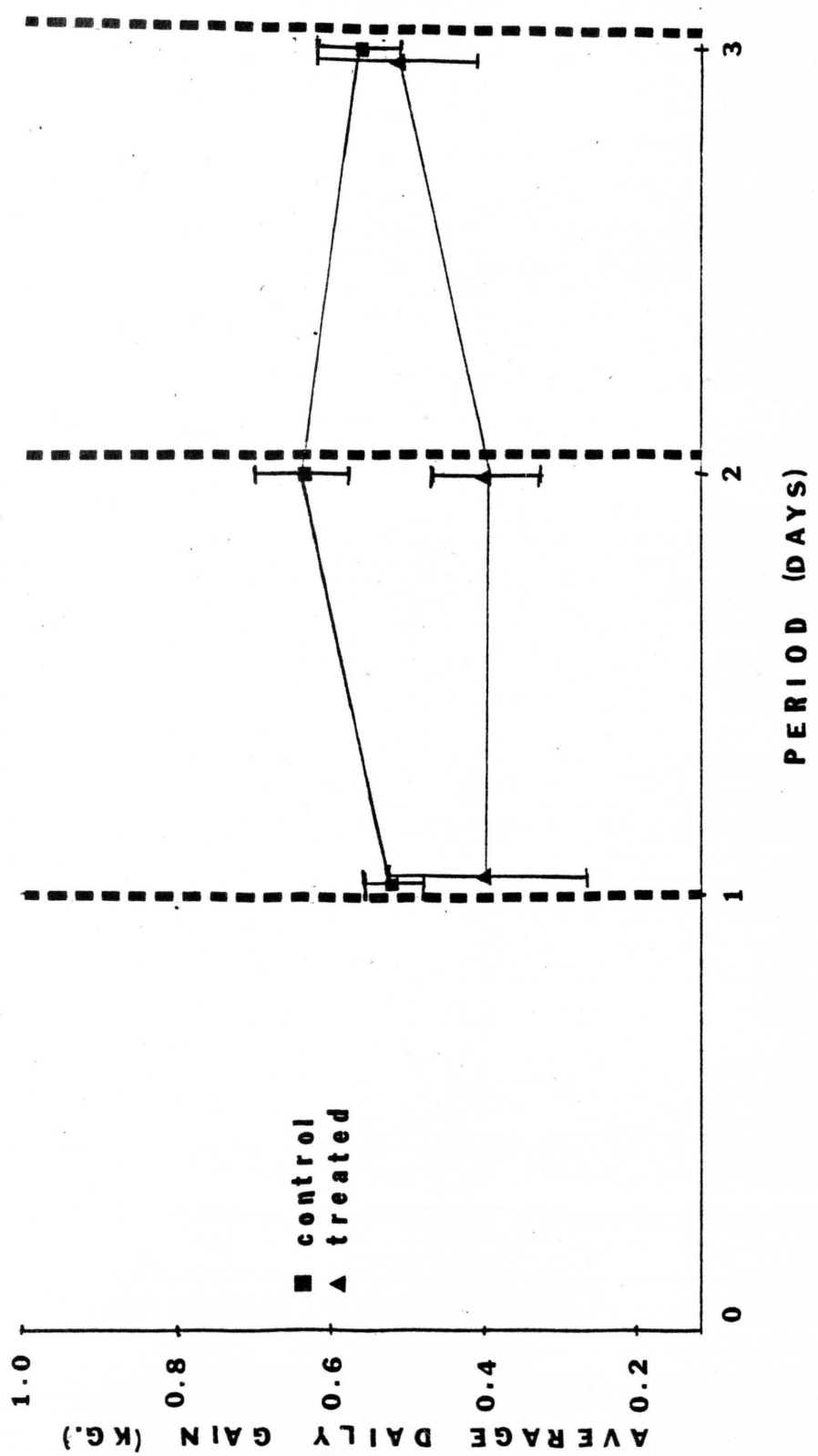
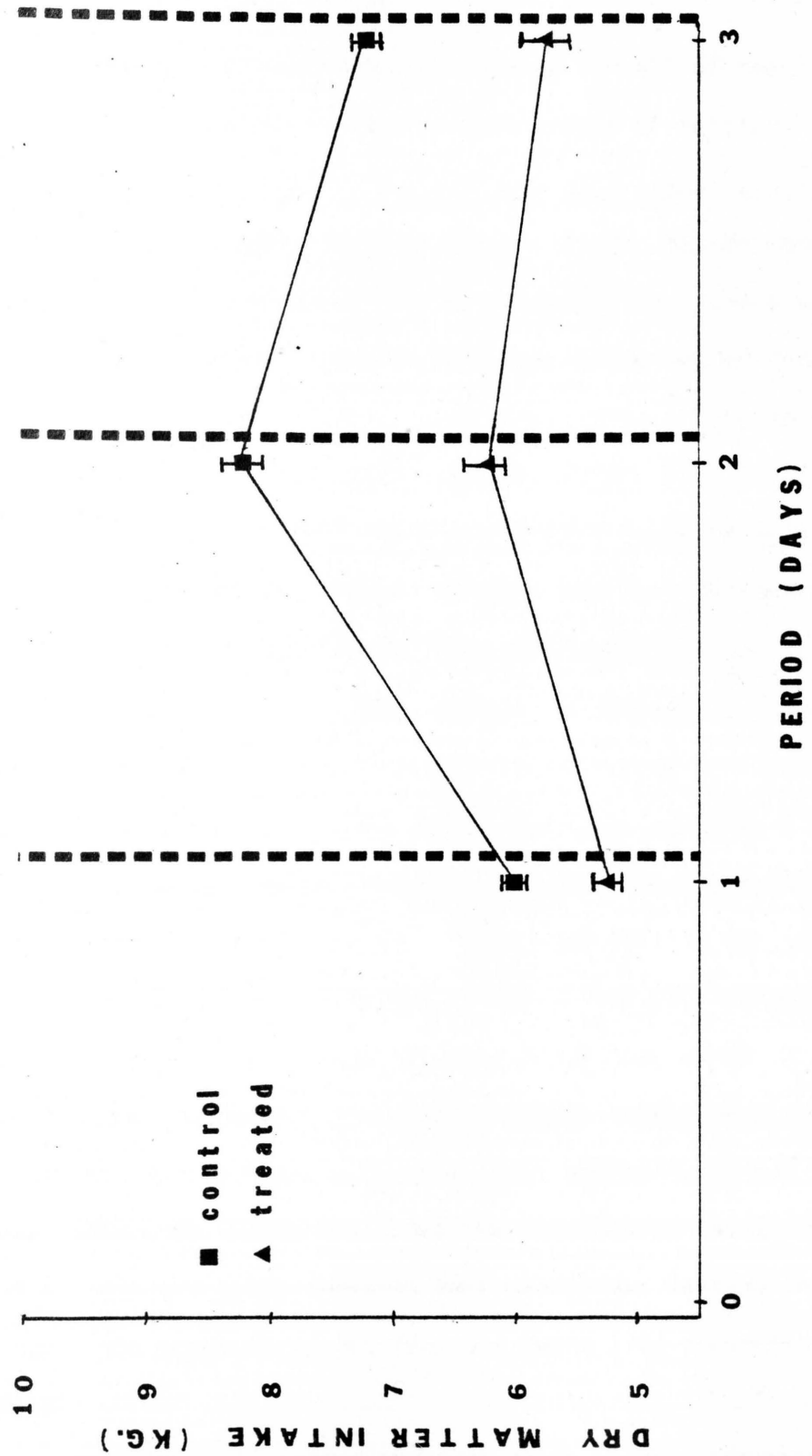

AVERAGE DAILY GAIN (KG.)
1.0
0.8
0.6
0.4
0.2
0
1
2
3
PERIOD (DAYS)
control
treated

Figure 5. Dry matter consumption of alfalfa haylage by heifers.


DRY MATTER INTAKE (KG.)
10
9
8
7
6
5
0
1
2
3
PERIOD (DAYS)
control
treated

heifers was 5.1 (control) and 7.6 kg (propionic acid). The consumption value recorded for the treated haylage was biased because one heifer fed the treated haylage consumed nearly three times as much high-phosphorus supplement block as the next highest consumption recorded for any other heifer. The same calf experienced diarrhea during the second week of the trial. Daily consumption of the high phosphorus block ranged from 2.5 to 6.3 (control) and from 3.4 to 15.8 kg (treated). Forages are generally inadequate in phosphorus content and are usually supplemented with phosphorus. Calves were supplemented with trace-mineral (TM) blocks. Average daily TM consumption for calves fed the control haylage ranged from 1.2 kg to 2.0 kg with a mean of 1.5 kg. Calves fed the treated haylage consumed an average of 1.3 kg with a range from 0.3 kg to 2.1 kg of trace-mineral block.

Composition of the haylage was generally not reflected in the rumen fluid. Calves fed the control haylage consumed more dry matter with slightly better gain and feed efficiency. Both haylages were not adequate in energy to support growth of dairy heifers.

CONCLUSIONS

Several conclusions were drawn from these experiments.

1. Covered haylage was superior to propionic acid treated haylage in quality as measured by haylage chemical composition and animal performance.
2. Covering and/or propionic acid addition, as in experiment 1, lowered ensiling temperature: covering was more effective than propionic acid in this respect. Addition of propionic acid, as in experiment 2, did not lower ensiling temperature as compared to the control.
3. Covering and depth of haylage in the silo generally had a major influence on chemical composition, whereas, propionic acid addition and length of storage had minor or no influence.
4. Regardless of treatment or cover, alfalfa haylage should be supplemented with energy if fed to dairy heifers weighing 150 to 250 kg.

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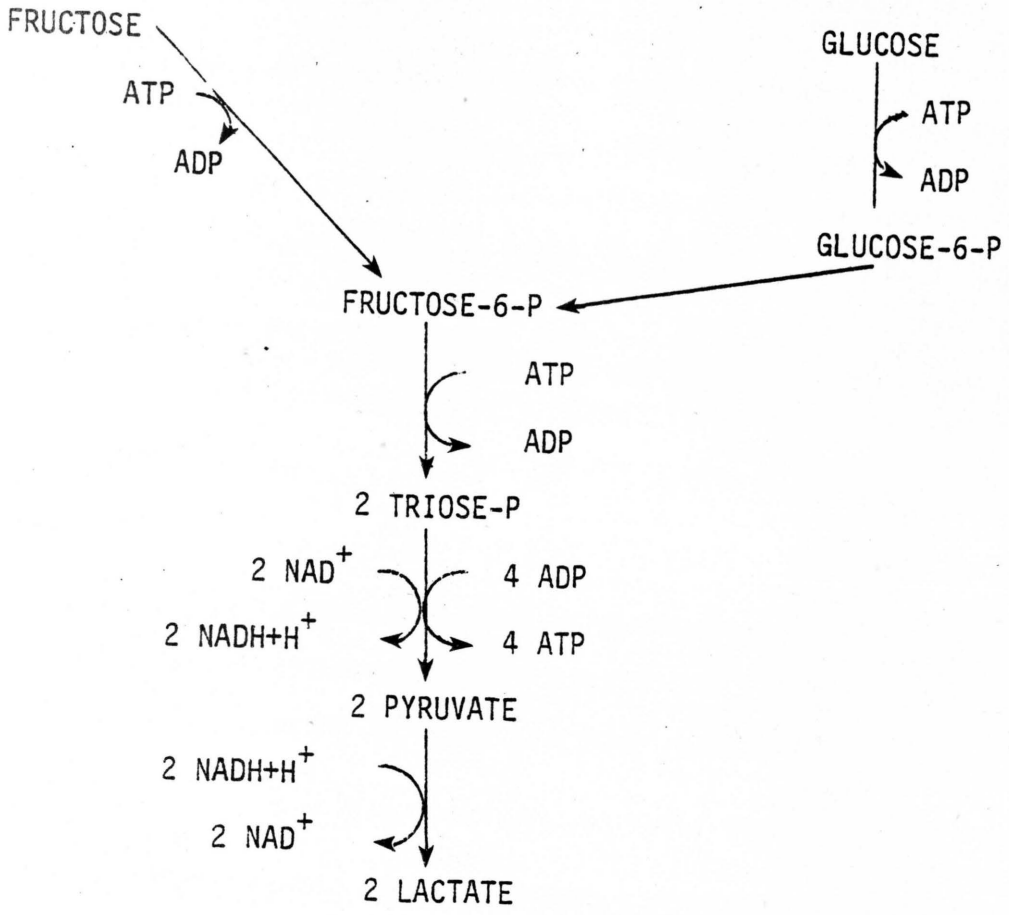
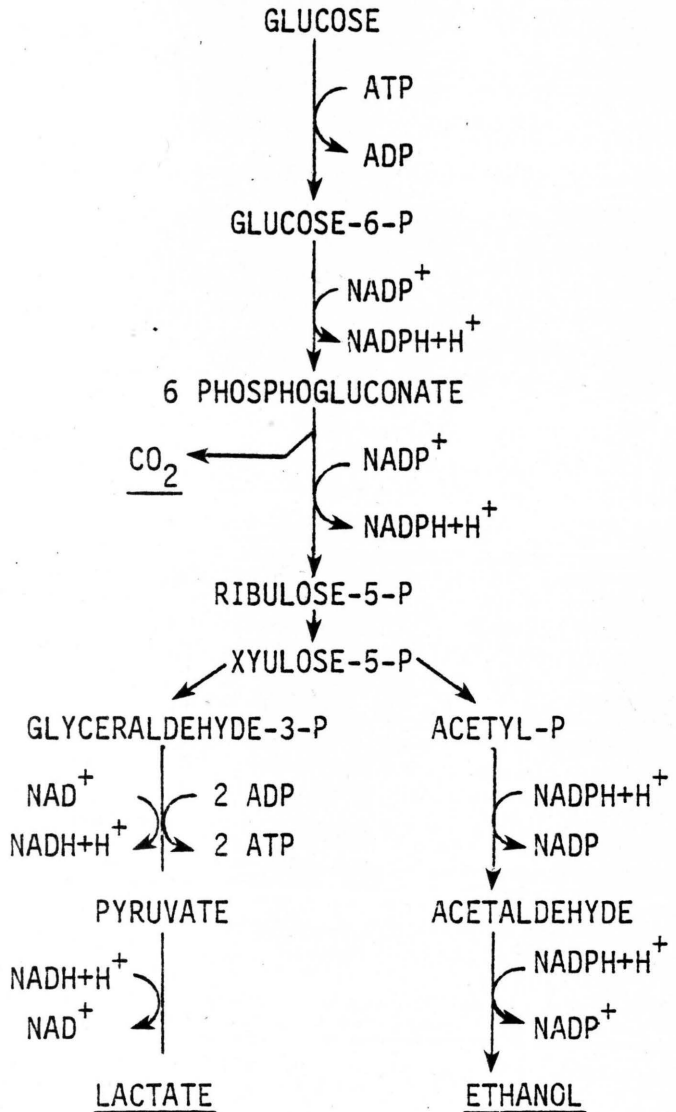
APPENDIX TABLE 1. Species of lactic acid producing bacteria commonly found in silage.

Homofermentative	Heterofermentative
Lactobacillus plantarum	*Lactobacillus brevis*
Pediococcus acidilactici	*Lactobacillus buchneri*
Streptococcus faecalis	*Lactobacillus fermentum*
Streptococcus faecium	*Lactobacillus viridescens*
Streptococcus lactis	*Leuconostoc mesenteroides*

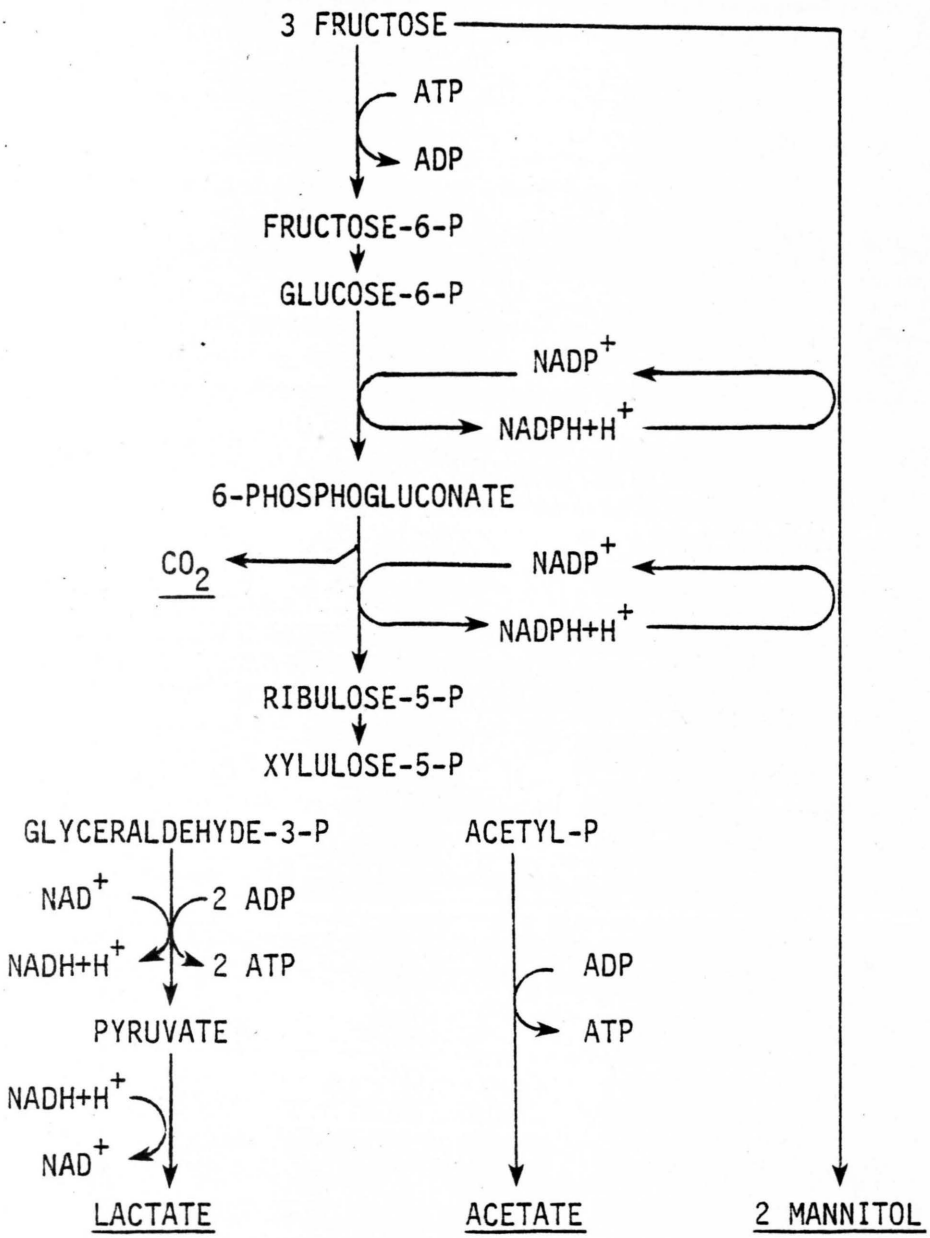
Appendix Figure 1. Homolactic fermentation of glucose and fructose.


FRUCTOSE
ATP
ADP
GLUCOSE
ATP
ADP
GLUCOSE-6-P
FRUCTOSE-6-P
ATP
ADP
2 TRIOSE-P
2 NAD+
4 ADP
2 NADH+H+
4 ATP
2 PYRUVATE
2 NADH+H+
2 NAD+
2 LACTATE

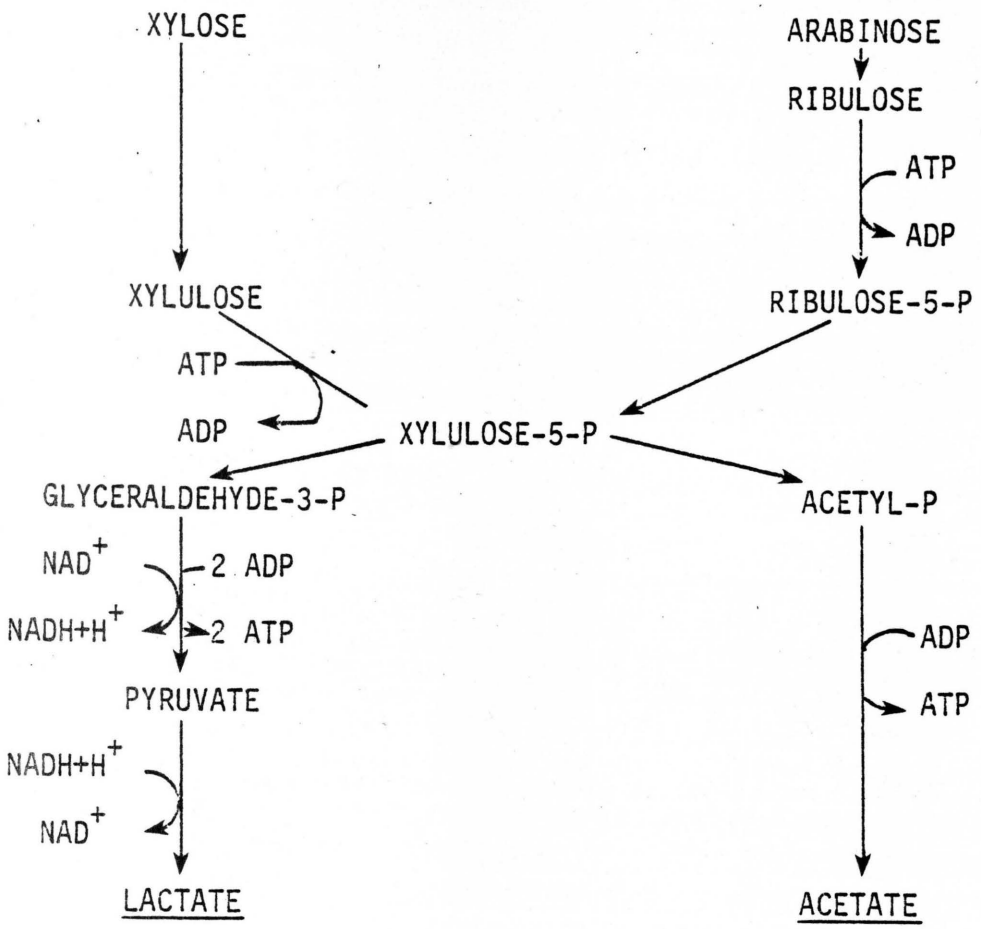
Appendix Figure 2. Heterolactic fermentation of glucose.


GLUCOSE
ATP
ADP
GLUCOSE-6-P
NADP+
NADPH+H+
6 PHOSPHOGLUCONATE
CO2
NADP+
NADPH+H+
RIBULOSE-5-P
XYULOSE-5-P
GLYCERALDEHYDE-3-P
ACETYL-P
NAD+
NADH+H+
2 ADP
2 ATP
NADPH+H+
NADP
PYRUVATE
ACETALDEHYDE
NADH+H+
NAD+
NADPH+H+
NADP+
LACTATE
ETHANOL

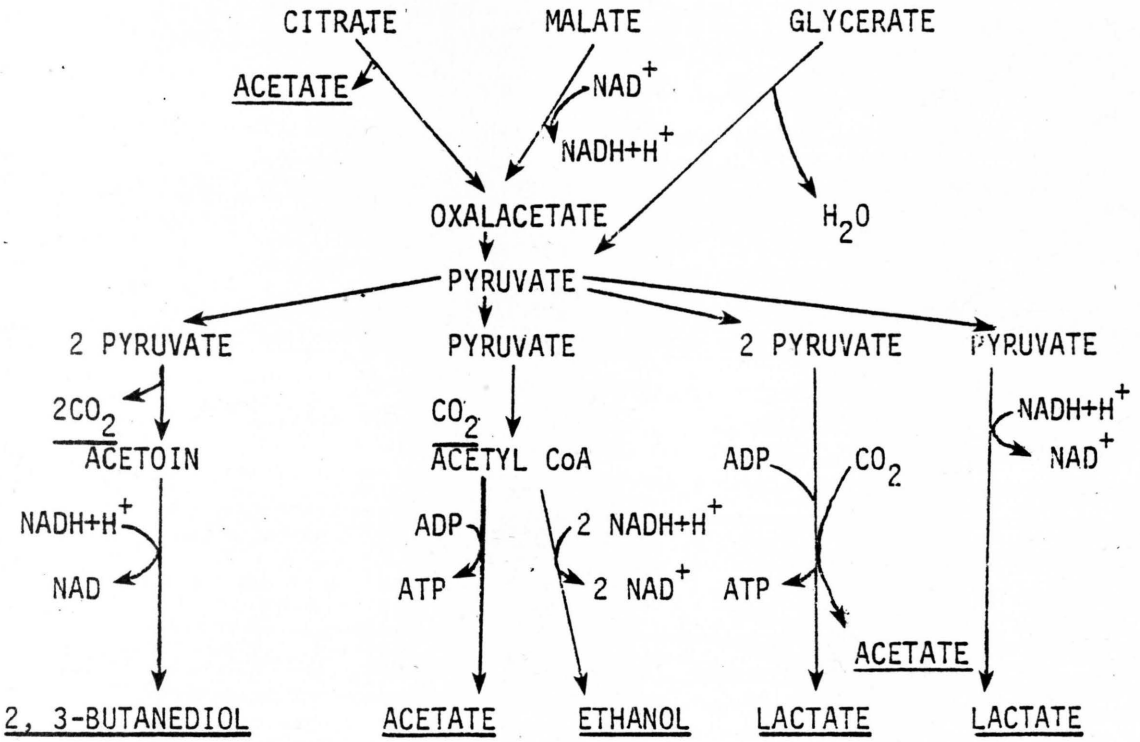
Appendix Figure 3. Heterolactic fermentation of fructose.


3 FRUCTOSE
ATP
ADP
FRUCTOSE-6-P
GLUCOSE-6-P
NADP+
NADPH+H+
6-PHOSPHOGLUCONATE
CO2
NADP+
NADPH+H+
RIBULOSE-5-P
XYLULOSE-5-P
GLYCERALDEHYDE-3-P
NAD+
2 ADP
NADH+H+
2 ATP
PYRUVATE
NADH+H+
NAD+
LACTATE
ACETYL-P
ADP
ATP
ACETATE
2 MANNITOL

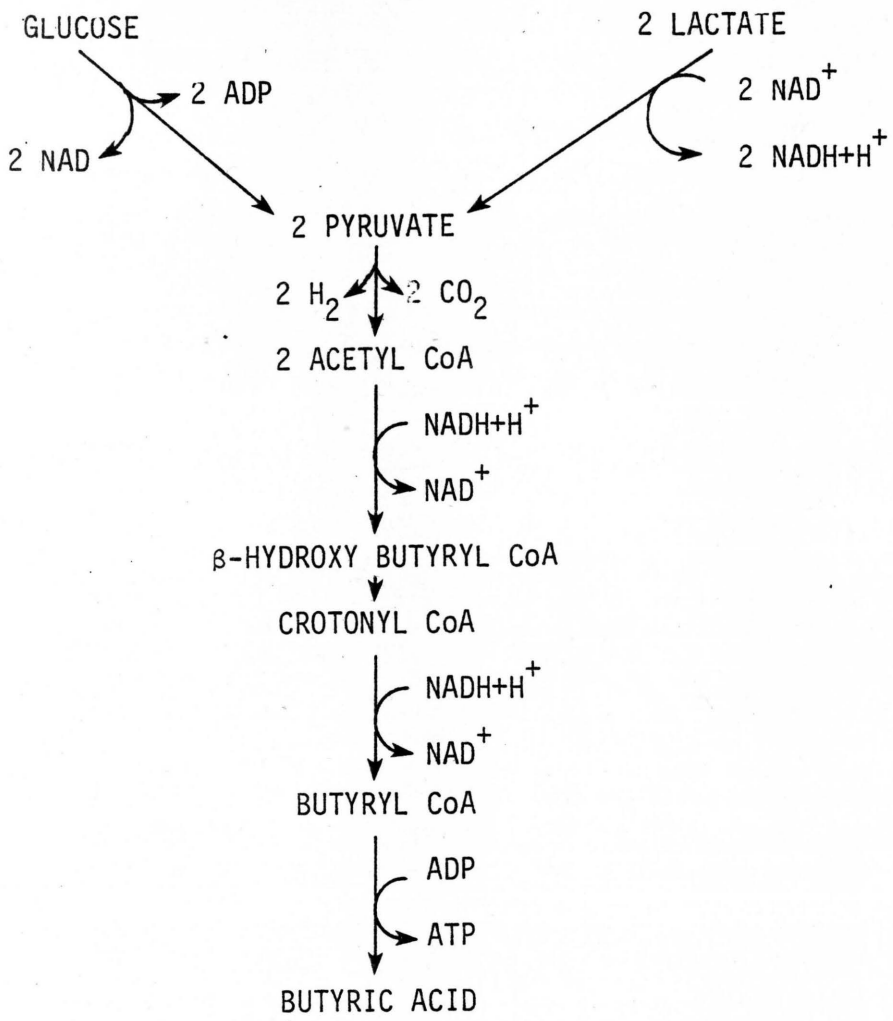
Appendix Figure 4. Fermentation of pentoses by lactic acid bacteria.


XYLOSE
ARABINOSE
RIBULOSE
ATP
ADP
XYLULOSE
RIBULOSE-5-P
ATP
ADP
XYLULOSE-5-P
GLYCERALDEHYDE-3-P
ACETYL-P
NAD+
2 ADP
NADH+H+
2 ATP
ADP
ATP
PYRUVATE
NADH+H+
NAD+
LACTATE
ACETATE

Appendix Figure 5. Fermentation of organic acids by lactic acid bacteria.


CITRATE
MALATE
GLYCERATE
ACETATE
NAD+
NADH+H+
OXALACETATE
H2O
PYRUVATE
2 PYRUVATE
PYRUVATE
2 PYRUVATE
PYRUVATE
2CO2
ACETOIN
CO2
ACETYL CoA
ADP
CO2
NADH+H+
NAD+
NADH+H+
NAD
ADP
ATP
2 NADH+H+
2 NAD+
ATP
ACETATE
2, 3-BUTANEDIOL
ACETATE
ETHANOL
LACTATE
LACTATE

Appendix Figure 6. Fermentation of glucose and lactate by saccharolytic clostridia.


GLUCOSE
2 ADP
2 NAD
2 LACTATE
2 NAD+
2 NADH+H+
2 PYRUVATE
2 H2
2 CO2
2 ACETYL CoA
NADH+H+
NAD+
β-HYDROXY BUTYRYL CoA
CROTONYL CoA
NADH+H+
NAD+
BUTYRYL CoA
ADP
ATP
BUTYRIC ACID