EFFECT OF ORGANIC ACIDS AND NON-PROTEIN-NITROGEN ON FUNGAL GROWTH, NUTRITIVE VALUE, FERMENTATION, AND REFERMENTATION OF CORN SILAGE AND HIGH MOISTURE CORN

Dissertation for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
DANNY GILBERT BRITT
1973

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presented by

Danny Gilbert Britt

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Dairy Science
and Institute of Nutrition

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Major professor

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ABSTRACT

EFFECT OF ORGANIC ACIDS AND NON-PROTEIN-NITROGEN ON FUNGAL GROWTH, NUTRITIVE VALUE, FERMENTATION AND REFERMENTATION OF CORN SILAGE AND HIGH MOISTURE CORN

By

Danny Gilbert Britt

Experiments were conducted with corn silage and high moisture shelled corn to evaluate the preservative value of organic acids and non-protein-nitrogen additives on the fungal growth during fermentation and refermentation.

Whole chopped corn (35% DM) in lots of 56 kg was treated with either propionic, formic, propionic plus formic or propionic plus acetic acids at 0, 0.5, 1 or 2%; and with either urea, aqua-ammonia or ammonia-molasses solution at 0, .2, .4, or .8% added nitrogen. Treated material was placed in polyethylene bags inside 200 ℓ drums, evacuated, and sealed. Forages were sampled and temperatures measured at various intervals during fermentation. After fermentation was completed, 12 kg portions of the preserved forages were placed in open containers and stored at 25 C. At various dates during refermentation forages were

sampled, aerated, and temperatures measured. Samples were analyzed for VFA, lactic acid, pH, and number and type of fungi.

During refermentation acid treatment at 2% reduced the average temperatures when compared to controls (19.8° vs 24.4°; $P < .01$); and .5 and 2% acid reduced ($P < .01$) the maximum temperature compared to controls (33.6°, 29.6°, and 37.5°, respectively). Propionic at .5 and 1% was more effective than other acids. Lactic acid production was significantly depressed by all acids at 2% addition. All treatments containing propionic acid required more days ($P < .01$) before visual mold was detected (7.0, 13.4, 20.0, and 20.5 days; for formic, propionic plus formic, propionic plus acetic and propionic, respectively). Days until complete molding was also increased by acid treatment. All acids significantly decreased fungal colonies within 2 days after addition. During refermentation all treatments at 1% acid exhibited a rapid increase in number of colonies; however, propionic acid treated silages showed less fungal growth than those treated with formic acid.

The relative proportion of yeasts was greatest at initiation of fermentation and decreased at day 40 (from 70 to 10%). During refermentation, growth of yeast again accelerated. The proportion of Geotrichum was highest at day 40 of fermentation. Aspergillus was significantly higher at day 40 of fermentation and day 36 of refermentation. No significant amounts of Penicillium were detected at any date.

Initial pH increased with nitrogen addition. At .8% nitrogen, pH values for urea, aqua-ammonia and ammonia-solution were 6.35, 9.88, and 9.65, respectively. Added nitrogen also resulted in large pH increases during refermentation. Ammonia added at .2% nitrogen increased, but higher levels depressed lactate production in silages. Upon spoilage lactic acid disappeared. Silages treated with the ammonia-solution required the longest to spoil ($P < .01$). Ammonia nitrogen reduced total fungal colony counts ($P < .01$) at 30 minutes after treatment; but during fermentation, no differences between treatments were noted. At both .4 and .8% nitrogen, fungal counts were lower ($P < .01$) than at 0 and .2%. Treatment did not significantly change the relative proportions of fungi during spoilage. The majority of fungi were yeast and Geotrichum while some Aspergillus and Penicillium were identified. However, yeast decreased and Geotrichum increased during spoilage on all treatments ($P < .01$).

High moisture shelled corn (27% moisture) was treated with: 1) propionic acid (at 1.2%), 2) a mixture of 80% propionic and 20% acetic acid (1.2%), 3) aqua-ammonia (at .54% NH_3) or 4) a commercial ammonia solution (at .63% NH_3). Calculated recovery of nitrogen for the ammonia treatments after storage was 60% on the ammonia solution and 36% for the aqua-ammonia while about 80% of the propionic acid was recovered. No significant changes in nitrogen or acid content were

observed during storage. Fungi were reduced by all additives (21 vs 690 colonies/g X 10^3) 30 minutes after treatment. Fungal colony counts after 28 days for the aqua-ammonia and control treatments were significantly higher than the others. During late storage both ammonia treatments showed increases in fungal colonies. They occurred earlier and were of greater magnitude for the aqua- than the ammonia-solution. After 60 days of storage, corn treated with aqua-ammonia heated to 50 C while the other treatments remained at ambient temperature. After rolling the corn prior to feeding the aqua-ammonia corn had higher fungi ($P < .05$) than other treatments and that treated with the ammonia-solution heated to 30 C. The propionic acid treated corn remained at ambient temperature (4-10 C) and did not heat after rolling.

Lactating dairy cows (8 per group) readily consumed all corn in a 5-week feeding trial even though that treated with aqua-ammonia was heavily molded. No health problems were detected in any of the cows due to eating the moldy corn. Milk production was 21.2, 18.4, and 19.3 kg for treatments 1, 3, and 4 respectively ($P < .109$) and persistencies of milk yield (trt/std) averaged 93, 88, and 81% ($P < .126$).

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By

Danny Gilbert Britt

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Dairy Science and Institute of Nutrition

1973

GR6559

DEDICATION

I would like to dedicate this thesis to my father, Mr. Gilbert Britt, and grandfather, Mr. Andrew Britt, who instilled in the author the desire to never quit.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. J. T. Huber for his advice and counsel throughout his graduate program. His support and encouragement have been greatly appreciated.

The author would also like to express his gratitude to other members of the graduate committee; Dr. H. Hafs, Dr. A. Rogers, and Dr. R. Luecke for their guidance and counsel during the author's graduate program.

Thanks are extended to Dr. C. A. Lassiter and Dr. J. A. Hoefer for making the facilities at Michigan State University available for this research and to Dr. J. W. Thomas for his assistance in obtaining financial support through NIH.

Appreciation is also extended to Alfred Dutrow, Richard Greening, Judy Ball, and Dr. Roger Neitzel for their help during this research.

The writer wishes to extend his sincere gratitude to his wife, Carolyn, and son, Danny Joe, for their sacrifices, encouragement, and assistance during his course of study and manuscript preparation.

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I. INTRODUCTION

Changes in recent years have been oriented toward the storage of corn silage, haylage and corn, at moistures more susceptible to fungal growth, especially after aeration upon removal from storage. These fungal contaminants can produce a wide variety of toxic metabolites called mycotoxins (Lynch, 1972). A survey conducted in the United States revealed no contamination of the milk supply (Brewington et al., 1970); however, Purchase and Vooster (1968) reported contamination of commercially available milk in South Africa.

Organic acids have been reported to be effective in preventing fungal growth (Sleiman, 1972; Richardson and Halick, 1957). In addition, Bothast et al. (1973) reported that ammonia may be used as a fungicide in stored grains. There is the possibility that these treatments may select for a more potent toxin forming fungi.

The objectives of this thesis were to examine the type and number of fungi which grew during fermentation and refermentation of non-protein nitrogen and organic acid treated corn silage and high moisture corn. The nutritive value of the treated HMC was also compared using lactating dairy cows.

II. REVIEW OF LITERATURE

Recently emphasis has been placed on treating forages to decrease losses during storage and prevent spoilage due to fungal growth. In addition much interest has developed in preserving high moisture corn (HMC) with volatile fatty acids (VFA).

This review will discuss the problem of mycotoxin formation in feedstuffs and the effect of chemical treatments on the fungal growth patterns.

Fungi Development in Feedstuffs

Role of Fungi in Forage and Grain Storage

The problem of fungal contamination of feedstuffs is twofold. One is the loss of nutrients in feeds due to fungal growth and the other involves secretion of metabolites which are toxic to animals. Until recently, most interest has been in the loss of nutrients. Recently, however, due to changes in harvesting methods and more sensitive analytical procedures, concern has developed over the possible

contamination of feeds with toxins, their effects on the animal, and entrance into the human food supply.

Wogan (1964) stated that ergotism has been known for centuries and occasional reports have appeared during the past three decades associating ingestion of mold-contaminated foodstuffs with a variety of toxicity syndromes in domestic animals and in one instance man. However there is still a failure to appreciate the significance of food-borne mycotoxins in problems of animal and human health.

Types of Fungi Present

Different types of fungi are present during harvesting and storage of grains. These are the field fungi and storage fungi. Tuite and Christensen (1955) found Alternaria, Cladosporium, and Fusarium were common in seeds prior to harvest while Aspergillus and Penicillium appeared after harvest. Christensen (1949) found that the common field fungi were Alternaria, Helminthosporium, and Fusarium while Aspergillus and Penicillium were the predominate storage fungi and grew best at about 30° C. In a survey of fungi in flour, Christensen and Cohen (1950) found counts ranging from several hundred to about 5,000 per gram. These counts remained stable for about 2 years and were predominately Aspergillus and Penicillium. Bothast and coworkers (1973) reported mold counts of whole corn varied from 10^2 to

10^6 in the 1970-1971 crop. Penicillium and Fusarium species predominated among the molds but Aspergillus, Helminthosporium, Nigrospora, and Trichoderma were significant.

Not all fungi which grow on grains are toxic. Scott (1965) isolated 228 strains from domestic cereal and legume crops and of these, 46 were toxic when grown in pure-culture and fed to Pekin ducklings. Christensen and co-workers (1968) isolated 943 fungi strains in 40 genera from feeds, peanut fruits and seeds of which 54% were toxic to rats within 7 days. Richard et al. (1969) isolated 246 fungi from 25 moldy corn samples in Iowa. Extracts of 99 of these isolates were toxic with the majority of the toxic isolates being Aspergillus or Penicillium. Burnside et al. (1957) isolated 13 cultures of mold from fields where hogs were dying. Two of the 13 cultures produced toxins. These were identified as Penicillium rubrum and Aspergillus flavus. Semeniuk et al. (1971) isolated 392 strains of Aspergillus and found 166 of these were toxigenic.

Role of Fungi in Heating of Feeds

Gilman and Barron (1930) tempered grain to 18% moisture and sterilized the grain. The grain was then allowed to germinate with or without fungal inoculation. The presence of fungi imcreased the temperature over the non-inoculated from 5.2 to 26.4 C depending on

type of grain. They concluded that there was a high probability that in bins of stored grains the marked increase in temperature may be ascribed to mold growth. Milner and Geddes (1946) reported that mold growth was responsible for soybean heating to 50-55 C and was associated with the growth of A. glaucus and A. flavus. Milner and Geddes (1945) found mold proliferation in seed, as visually observed, was positively correlated with respiratory activity, and with increases in temperature to 40 C.

In further support Christensen et al. (1949) reported a close correlation between increase in production of CO_2 and mold population. Christensen and Gordon (1948) found molds caused the temperature to rise within a few degrees of the maximum that the molds could endure. Autoclaved, moist wheat inoculated with 200,000 spores of A. flavus per gram heated to 45 C in two days while that inoculated with 0.2 spore per gram took nine days to heat to a comparable temperature. They concluded that the amount of inoculum originally present has only a minor effect on eventual heating.

Honig (1969) conducted gas balance tests with silages and found DM losses increased linearly with the amount of added air. The digestibility of nutrients as well as the quality and stability of silages decreased with increased air. Because fungi are aerobic this air may have been stimulating fungal growth but this was not mentioned. In support, Federson (1971) found oxidation of high

moisture silages in insulated silos was accompanied by a large rise in temperature and high DM losses. Temperature increases in the silos without oxygen present were negligible. He observed that the pH closely paralleled the added oxygen. Gordon (1968) harvested corn silage at late maturity (58-63% DM) and early maturity (26-30% DM) and found digestibility of DM and acid detergent fiber were lower in the mature silage. Early silage had more VFA and lactic acid and the late silage had a tendency to heat when fed in hot weather.

Gregory and co-workers (1963) harvested wet hay (60% DM) which became very hot and contained a large flora of thermophilic fungi. Actinomycetes and bacteria grew during the first heating with increases in acidity and volatile nitrogen. When fungi grew the pH rose to 7.0 or above. The wet stack developed a brown acid hay center containing many spore-forming bacteria but few fungi, surrounded by an outside layer of moldy hay. According to Thomas and Hillman (1972), excessive heating during curing of haylage, baled hay or stacked hay caused carmelization to occur between plant proteins, sugar and water resulting in a product which was insoluble and indigestible. They concluded the carmelization effect was small but measurable when forages heated to 46C, greater at 51.7 C and protein digestibility was markedly reduced at 57 C. Perry et al. (1968) suggested that the corn plant may be harvested at a much later stage than hard dough

which has been recommended by other workers if stored in gas-tight silos which would keep fungal contamination to a minimum.

Mycotoxin Production by Fungi During Feed Storage

Conditions under which Toxins Are Produced

A complete listing of all toxins produced by fungi is too lengthy for this review, but such a list has been published by Wasserman (1968) in which he listed 11 toxins produced by Alternaria, 16 by Fusarium, 66 by Aspergillus and 98 by Penicillium. Crane et al. (1972) suggested four conditions for aflatoxin production. These were: 1) suitable strain of fungi, 2) correct moisture and relative humidity, 3) optimum temperature, and 4) sufficient time in storage.

Koehler (1938) reported Aspergillus grew at a minimum of 14.3% moisture, Penicillium at 15.6 to 20.8 and Fusarium moniliforme at 18.4 to 21.2%. Trenk and Hartman (1968) concluded that rewet corn at moistures below 17.5% and temperatures below 13 C was not susceptible to aflatoxin formation by A. flavus. From 19 to 28% moisture, toxin content increased linearly from 50 to 2,000 PPB.

Diener and Davis (1967) reported the maximum temperature for aflatoxin production was 41.5 C, the limiting relative humidity was

85% and minimum temperature was 13 C. Milner and Geddes (1946) concluded the relative humidity rather than actual moisture determine susceptibility to molding. The critical moisture values for different seed species are those in equilibrium with a relative humidity of about 75%.

Lutey and Christensen (1963) found that storage of barley at 15% moisture and 30 C for 16 weeks resulted in total loss of viability of all field fungi and little invasion of Aspergillus. Landecker and Stotzky (1972) found that when they grew fungi in the presence of bacteria, the bacteria strongly inhibited colony spread and sporulation. Moreover, colony morphology was changed presumably by the release of volatile metabolites.

Using casein substrates, Lie and Marth (1968) found A. flavus was able to initiate growth in the pH range of 1.7 to 9.34, with the best growth between 3.42 and 5.47 mold growth was always associated with a pH change toward neutrality. Both aflatoxins B_1 and G_1 were detected in all samples which supported mold growth, but highest concentrations were noted at the pH extremes.

Documented Cases of
Mytotoxin Poisoning

When feeds are subjected to fungal growth, the fungi produces metabolites which may be beneficial, harmful or have no effect on the animals which consume the moldy feed. Christensen and co-workers (1973) added a toxin-producing strain of Aspergillus flavus to grain which was heavily infested with natural fungi. A small amount of toxin was found in one sample, but feeding this grain caused no injury in rats, ducklings or broiler chicks. This work suggests that the danger of toxicity from materials invaded by a mixture of fungi, including one strain of A. flavus capable of producing toxin, is not great.

Whiteher (1971), Doupnik and co-workers (1971) and Seerley and co-workers (1972) fed corn infected with Helminthosporium maydis and found a complete lack of toxicity in swine, chicks, mice and rabbits. They did note a decreased digestibility and cautioned that this blighted corn was more susceptible to invading fungi during storage and care should be taken to avoid secondary contamination.

In feeding an extract of Aspergillus oryzae to sheep, Niver, Tucker and Mitchell (1971) found no effect on fiber digestibility or N balance and defaunation of the rumen was not detected. Hintz and co-workers (1967) fed 450 PPB of aflatoxin B_1 to pigs from 3 to 8 months of age with no harmful effects. All gilts and one boar were

continued on feed for a reproductive study which resulted in all offsprings appearing normal at 6 weeks of age.

Lynch (1972) listed the main storage fungi and toxins produced:

Aspergilli - Aflatoxins, ochratoxins, patulin and sterigmatocystin

Fusaria - Zearalenone, acetamido lactone, tricothecenes

Penicillia - Rubratoxins, patulin, citrinnin and tremorgenic factors

Other - Slaframine, sporidesmins, ergots

Mirocha et al. (1968) reported that when moldy hay was included in the ration of 150 dairy cattle the number of services per conception jumped from 1.2 to 4.0. An estrogenic factor was isolated (F-2) at a concentration of 14 PPM. When cultured, Aspergillus sp. and Penicillium sp. were found but Fusarium would have died off at moisture contents that permit growth of Aspergillus and Penicillium. Burt and coworkers (1964) reported that when hay which was not visually molded but had total spore counts of 2,000,000/g was included in the ration of dairy cows, a 6% decline in milk yield was observed. Large numbers of Aspergillus and Penicillium were detected in this hay.

Lynch et al. (1969) reported a field case in which ensiled, moldy, shelled corn produced tetany symptoms in milk cows. Of the four cases which occurred, all responded to IV infusion of calcium gluconate. Apparently this ration contained a normal level of minerals.

The most predominate fungi in the corn were yeasts, Mucors, Penicillia, Monascus, and Byssochlamys.

Still et al. (1971) isolated Aspergillus ochraceus from moldy hay that was associated with abortions in dairy cattle. Ochratoxin was isolated from a pure culture of the fungi and caused faetal death in rats. Albright and co-workers (1964) diagnosed a gross hemorrhagic syndrome in 20 to 29 heifers fed a ration containing toxin-producing strains of Aspergillus flavus, Penicillium cyclopium and Penicillium palitans. Smith and Lynch (1973) analyzed mold and unmoldy corn silage which had been fed to a herd of cows in which several deaths had occurred. They found A. fumigatus at dilutions of 5×10^6, but analysis of feed for toxins by thin-layer chromatography was negative. They concluded that the corn silage may or may not have been implicated in the death of the cows.

Mohanty et al. (1969) molded alfalfa hay by sprinkling with water. They hay quickly heated to 57-64 C. Molding lowered DM, ether extract, and NFE, while increasing pH, ash and ammoniacal nitrogen. In feeding trials, the molded hay reduced DM intake, BW gains, total VFA, ruminal ammonia, and rumen protozoal counts; whereas, increases in rumen pH, percent acetate and Diplodinium numbers were noted. Steers fed the molded hay showed laxation of feces and developed rough hair coats. Nineteen mold species were isolated with Scopulariopsis brevicalis predominant.

Mohanty et al. (1968) in another report molded hay and extracted with various solvents. The chloroform extract gave the greatest growth depression when fed to baby chicks. Aspergillus appeared to be predominate fungal species in this study.

In ewes Cysewski and Pier (1968) produced abortions with IV injections of spores from A. fumigatus. Chu and Chung (1971) reported the L D_{50} in day-old chicks to be 166 and 216 µg for ochratoxin A and C. No toxic effect was demonstrated when chicks were fed with up to 500 µg/chick of dihydroisocoumarin, the hydrolyzed product. Christensen and co-workers (1965) found that 12 of 85 isolates of Fusarium caused increases of 5-8 times in weight of the uterus of rats. These isolates originated from feed collected on farms.

Kurtz et al. (1969) reported gilts given estradiol (F-2) mycotoxin and corn which had been inoculated with Fusarium graminearium exhibited the same histological changes in the genital tract. These were characterized by squamous cell metaplasia and loss of normal mucosal epithelium off the vagina and cervix. Sharda et al. (1971) fed corn which had been inoculated with Fusarium rosium Ohio isolate C and showed it was highly toxic to rats, mice, hamsters, rabbits, and pigs. Post-mortem examination revealed jaundice, histological changes in liver and myocardial granulomas. Molds depressed feed consumption of all animals but pigs. Permanent damage did not appear in rats and replacement of moldy corn with good corn reversed symptoms.

Muller et al. (1970) reported sensitivities of several animals to aflatoxin. These animals in order of decreasing sensitivity were ducklings, turkey poults, goslings, young pheasants and chicks. Sinnhuber et al. (1969) has reported cancer formation in trout due to aflatoxin consumption. Krogh et al. (1970) isolated two nephrotic compounds, citrinin and oxalic acid from Penicillium viridicatum.

In addition to these toxins, Crump et al. (1963) reported isolation of Rhizotonia leguminicola from hay known to cause slobbering in cows. The isolate was grown in pure culture and fed to rats and guinea pigs and produced similar symptoms. Rainey et al. (1965) isolated a salivary factor from a pure culture of R. leguminicola which possessed parasympathomimetic action.

Toxicity and Secretion in Animals Given a Known Amount of Mycotoxin

Lynch et al. (1970) fed aflatoxin to six pairs of calves for 6 weeks. The calves exhibited a strong objection to feed containing more than .020 mg aflatoxin B_1. Weight changes, albumin/globulin ratios and total serum protein content of calves were not affected, but a significant increase in serum alkaline phosphatase occurred at .020 mg B_1/kg BW. At post mortem, livers showed a loss of color and adrenal hyperplasia was noted. Histological studies of the liver

indicated bile duct and central vein proliferation, accumulation of fat, and loss of glycogen. To avoid the feed refusal Lynch et al. (1971) dosed seven pairs of young dairy calves with 0 to .10 mg/kg BW of G_1 aflatoxin for 6 weeks. Most of the aflatoxin-induced changes occurred at the .08 and .10 mg doses by the second week of treatment. These were decreases in feed intake, gains, serum carotene, inorganic phosphorus, serum vit A, and increase serum alkaline phosphatase and total bilirubin. At post-mortem, livers were again light tan with bile duct proliferation at the .04 mg level. Gall bladders were 10 times their normal size.

Marth (1967) found rats excreted some aflatoxin as CO_2, some in urine and feces, but 6-9% of ingested toxin remained in the liver. In addition rats and dairy cattle can modify and excrete some toxin in the casein fraction of their milk.

Masri et al. (1967) fed a cow and a ewe aflatoxin B_1 in their diet. About 0.3% of the ingested aflatoxin appeared in the milk. From 2 to 3 percent of the original aflatoxin dose appeared as M_1 in milk, and excretion was completed a few hours after ingestion. Allcroft and co-workers (1968) gave a lactating cow an oral dose of 300 mg mixed aflatoxins (B_1, 44%; G_1, 44%; B_2, 2%). Urine, milk and feces was collected for 9 days. About 85% of the toxin secreted was detected in 48 hours; however, only 4.5% of the original dose was recovered. The milk had .18% as M_1, the urine 1.55% as M_1 and 2.79%

was found in feces as B_1. Some G_1 was in both urine and feces. Apparently the cow must metabolize the toxins to M_1 before secretion.

The ewe reacts similarly to the cow in excretion of aflatoxins. Nabney et al. (1967) dosed a ewe with mixed aflatoxins (B_1, 36%; G_1, 52%; B_2, 3%; G_2, 2%). Ninety percent of that excreted occurred during the first 48 hours. Only 8.1% of the dose was recovered. Milk had 0.1% as M_1, urine 6.4% as M_1 and G_1, and feces 1.6% as G_1 and B_1.

Contamination of Human Food Supply Via Fungal Toxin Consumption by Animals

In a survey of commercially available milk in the United States no aflatoxin M_1, was detected (Brewington et al., 1970). In contrast Purchase and Vooster (1968) showed that 2 of 21 commercially available milk samples in South Africa contained aflatoxin M_1 in easily detectable amounts, while 3 others contained traces.

Methods of Controlling Mycotoxins in Feeds

Methods of preventing mycotoxin contamination of the human food chain are to destroy the toxins by chemical or physical means or prevent the toxin-producing fungi from developing.

Destruction of Mytoxins by Treatment

Dollear et al. (1968) heated peanut meal containing aflatoxins in the presence of moisture and chemicals. They showed that ammonia, methylamine, sodium hydroxide and ozone were effective in either destroying or reducing aflatoxin levels. Burnside et al. (1957) found the toxicity of mycotoxins unchanged after heating to 60 to 70 C for 26 hours. From these limited data, it appears that the most economical way to solve the mycotoxin problem is the prevention of fungal growth by chemical or physical treatment.

Prevention of Fungal Growth with a Fungicide

As early as 1945 Snow and Watts (1945) tested 50 isolates of molds for their reaction to sulphonilamide, sulphonamide E.O.S., sulphapyridine, sulphamezathine, sulphaquanidine, propamidine, and

phenamidine. They found sulphonamide doubled the storage life of ground linseed cake. Milner et al. (1947) tested the fungistatic ability of 100 compounds on wheat stored at 16 to 25% moisture. They found eight compounds which had satisfactory fungistatic properties. These compounds listed in order of their effectiveness are 8-hydroxyquinoline sulfate, thiourea, P-aminobenzoic acid, sulfonilamide, benezene sulfonamide, 2 aminothiozole chloramine B and calcium propionate. They found a variation in effectiveness for different fungi and suggested that a given compound should be tested on the flora of the product which is to be treated.

Christensen and Gordon (1948) found chloramine B, spergon dust, P-toluensulfonilamide or thiourea had very little fungicidal activity when applied to wheat inoculated with A. flavus. Knodt et al. (1952) reported sulfur dioxide treatment of wet forage produced a very palatable grass silage. The silage had decreased surface losses and spoilage rate during summer feeding.

Wittwer et al. (1955) treated red clover and grass forage with molasses, brewers dried grains and sodium bisulfite. Molasses-treated silage was most palatable and sodium bisulfite the least. They concluded that the small savings in nutrients could not justify the cost of preservatives. Srinivasan and Majunder (1965) reported that fumigation of kafir corn with methyl bromide and ethylene dibromide destroyed mold and bacteria.

Wilcox (1972) published a list of common feed preservatives and their characteristics:

Propionic acid	- very effective and widely tested preservative
	- pungent odor
	- calcium and sodium salts appear less effective
Acetic acid	- about 50% as effective as propionic at 16 to 20% moisture levels and less effective over 25%
	- more effective as a preservative when combined with propionic acid
	- much used in foods for pickling
	- pungent odor
	- salts of acetic acid have almost no preserving effect
Formic acid	- preserving effect somewhat less than acetic acid
	- dangerously caustic to skin
	- very pungent odor
	- calcium formate used in silages
Lactic acid	- some preserving effect in high moisture feeds
	- needs further testing as a grain preservative
Sorbic acid	- along with potassium sorbate is used in bakery doughs to inhibit molds and yeast
	- effective grain preservative when dissolved in alcohol and thoroughly distributed in the grain
Insobutyric acid	- reported to have preserving effect on grain
	- quite pungent odor

Benzoic acid and Sodium Benzoate - preservative for foods and fats
- limited by law to less than .1% in foods and feeds
- needs further testing for grain preservation

Propyl-p-hydroxy benzoate - used in foods--limited to not over .05%
- needs further testing for grain preservative effects

It appears that several compounds could be used as fungicides in feeds; but because of cost and legal restrictions the volatile fatty acids and their salts offer greatest promise. If their use is to become widespread they must not decrease the animal efficiency, but they must preserve the feed. Literature concerning the effect of these compounds on the animal and preservation of feeds will be reviewed.

Acid Treatment of Feeds and Grains

Acid Effect on Animal and Feed Quality

Simkins, Suttic and Baumgardt (1965) infused VFA mixtures (60% acetate, 20% propionate, 20% butyrate) into cows on pelleted alfalfa hay rations to meet 15% of the estimated digestible energy requirement and showed that infusion of propionic and butyric acids depressed feed intakes. They concluded that VFA's can act as satiety signal compounds.

Also, Montgomery et al. (1963) reported a significant decrease in hay consumption after intraruminal acetic acid infusion, while the infusion of propionic, butyric and lactic acids caused a moderate decrease in voluntary intakes.

Ulyatt (1965) reported decreased feed intakes in sheep intraruminally infused with 200 cal of acetic acid on low and high planes of nutrition, but the decrease was more pronounced on the low plane. Increased intakes resulted from infusing 200 cal. of propionic acid on both planes of nutrition; but at 300 cal. propionic depressed consumption at the low level of nutrition.

Bentley et al. (1956) reported the addition of sodium salts of acetic, propionic, and lactic acids to corn-cerelose-urea-hay or corn-hay rations produced significant increases in gains of lambs. The apparent feed replacement values were calculated at 1 kg of the acid salt for 3-10 kg of feed. Armstrong and Blaxter (1957) reported that the administration of 400 cal of acetic acid, propionic or n-butyric or 800 cal as n-buryric to sheep in positive energy balance did not interfere with the normal process of rumen fermentation or impose non-physiological conditions upon the animals.

Balch and Rowland (1959) administered .5 to 1.5 kg sodium acetate to cows on a fat-depressing ration and increased milk fat percentages. Administration of 414 g sodium propionate did not restore the fat percent and sodium acetate addition, to a normal diet, had no

affect on milk fat. Rook and Balch (1961) infused acetic, propionic and butyric acids intraruminally and reported acetic acid caused an increase in milk production and in yields of fat, lactose and protein, as well as an increase in fat percentage. Butyric or propionic acids had no effect on milk yield, but propionic decreased fat yields and percent while increasing percent protein and solid non-fat; whereas, butyric acid specifically increased the yield and percentage of fat.

Vercoe and Blaxter (1965) reported that the infusion of sheep with formic acid on dried grass diets at a constant rate for 17 days increased methane and CO_2 production but there was no significant change in O_2 consumption.

Birdson (1972) reported 6% faster gains in steers fed HMC treated with 1.5% acetic and propionic acids (60:40 ratio) compared to dry corn. Feed efficiencies favored the acid-treated corn. Jones et al. (1970) treated HMC with 1.5% propionic acid and ensiled other HMC. When the treated corn was fed to dairy cows and heifers FCM, persistency of milk production, milk fat and protein percent and rate of gain were not significantly different for animals fed untreated HMC.

Clark et al. (1973) fed 50 cows dry ensiled or propionic-treated corn with either hay or haylage. Cows fed ensiled and propionic-treated corn produced more 4% FCM than cows fed dry corn. In contrast to this Johnson and Otterby (1973) reported that

unadjusted milk production was higher on dry than high moisture or acid-preserved corn.

McCaffree and Merrill (1968) reported two trials with dairy cows in early lactation which were fed HMC. Feeding HMC resulted in lower forage and total DM intakes, milk fat percent, but higher actual milk production compared to feeding dry corn. Solids corrected milk was not affected. Barrington and Jorgensen (1971) found milk production and feed efficiencies were similar for HMC and dry corn, but milk fat tests were lower for HMC. In contrast to the drop in fat percentage usually seen with HMC feeding Johnson and Otterby (1971) reported that milk fat depression was not a problem in rations containing 33% HMC and corn silage.

Beeson and Perry (1958) found fattening beef cattle utilized high moisture ground ear corn (32% moisture) from 10-15% more efficiently than regular ground ear corn when grains were adjusted to the same moisture. In agreement with this Burroughs (1971) reported corn harvested and stored at 24-30% moisture had a feeding value on a DM basis 4-9% higher than artificially dried corn. Wilson and Long (1972) treated HMC with 1.6% acetic acid and propionic acids (60:40) and observed a 5% increase in feed efficiency of steers compared to steers fed dry-untreated corn. No significant differences in digestibility were seen.

Bayley et al. (1972) reported greater nitrogen and energy retention for high moisture corn preserved by addition of organic acids or by ensiling than dry corn in trials with pigs.

Bade et al. (1973) reconstituted dry sorghum to 70% DM with either water or 2% acid. Coefficients of digestibility for all components were highest for the water-reconstituted grain ration and lowest for the dry grain ration. Fat corrected milk was not affected by treatment but actual milk was highest on the reconstituted grain. Bolsen et al. (1973) tested ammonium isobutyrate, aureomycin, sodium hydroxide and a mixture of acetic and propionic acids as forage sorghum additives. The concluded that feeding values of forage sorghum silage was not significantly improved by any of the four additives.

Marion et al. (1972) fed steers HM sorghum grain treated with 0, 4, and 6% acetic or propionic acids. Steers gained well at the lower levels of acid and gains were higher than controls at 4%, and lower at 6% added acid. In another trial propionic acid was added at 2% to dry or reconstituted grain (30% moisture) and stored for 14 days in open barrels. When compared to the untreated grain no significant differences in daily gain, feed intakes, or efficiency were observed.

Factors Affecting Silage Quality

Barnett and Duncan (1954) and Langston et al. (1958) characterized poor quality silage by pH values of 4.8 or above, low amounts of lactic acid, high levels of butyric acid, and high ammonical nitrogen. Poor chemical quality and a lower rate of nutrient preservation were associated with poor quality silage. They recommended compressing the mass to make it more air tight.

Langston et al. (1962) observed aerated silages had high temperature and pH values with increased butyric acid and depressed lactic acid. They noted high total acids in sealed containers and concluded that high levels of sugar did not insure silage of superior quality unless the forage was packed properly to exclude air. Zimmer and Gordon (1964) using laboratory silos sealed for 38 days or sealed with the exception of aeration on days 1, 2, 3, and 6, reported higher O_2 consumption of unwilted, chopped silage during day 1 and 2 of aeration. Grinding the material improved total preservation and reduced CO_2 and DM losses. A correlation coefficient of +.71 was observed between CO_2 production and DM losses, so they concluded aeration resulted in poor preservation of the silage.

Wierginga et al. (1961) concluded that the presence of oxygen resulted in a faster loss of soluble sugars because respiration continues for a longer time. Above 40 C, oxygen was responsible for the fixation of protein into indigestible compounds. This temperature

was also associated with the highest percent of butyric acid. They recommended that silages be kept below 30 C to prevent putrefaction and fixation of protein which makes it indigestible.

Lopez et al. (1970) observed greater pH values for corn silages at low (25%) and high (52%) than at medium DM (30%). A significant decline in lactic acid concentrations was observed with advancing maturity. Total organic acids declined from 11.94% of DM in 25% DM to 3.14% of DM at 52%.

Ohyam et al. (1973) investigated the effect of glucose and temperature in grass silage preserved in laboratory jars. With no additive silage at 30 C was of very poor quality while at 15 C quality was improved. Glucose addition resulted in excellent quality silage irrespective of the temperature. Extent of protein breakdown was affected by temperature at the early stages and by pH at the later stages of ensiling.

Huber et al. (1972) treated corn silage with formic, acetic, propionic, and lactic acids at levels from .17% to .85%. Lactic acid production was depressed by formic acid (from 10% of DM in control to .9% on .85% formic), unchanged by acetic and increased at higher levels of propionic and lactic acid. Acetic acid levels were higher than control at .17, .34, and .57% formic, but 50% of the control at .85%. Acetic acid treatment increased silage acetate when .68 and .85% was added; whereas propionic and lactic acid additions decreased acetate

to about 50% of the controls. Huber (1970) also showed a greater depression in lactic acid content resulted from formic acid treatment of corn silage than has usually been reported in other crops (Castle and Watson, 1970).

Carpintero et al. (1969) treated lucerne with .85% formic acid and found this level was sufficient to achieve an immediate pH fall to 4.2. Formic acid inhibited both lactic acid and clostridial activities. They also observed preservation of water soluble carbohydrates (WSC) and suggested a beneficial effect on ruminants. Wilkins and Wilson (1969) added formic acid at the rate of one half gallon per ton to grass silage and detected an immediate drop in pH to 4.4 with little change during the first 12 days. Lactic acid in the treated silage was low.

Henderson and McDonald (1971) treated grass of low DM (11.8-17.3%) with formic acid and found the acid prevented oxidation of WSC. Treatment at .34% or higher also decreased proleotysis, lactic acid production and volatile nitrogen.

Castle and Watson (1970) treated timothy and perennial rye grasses (17-20% DM) with 0.2% formic acid. Formic acid treatments had about 7-15 C lower temperatures. Lactic acid was higher and butyric lower in treated silages indicating that formic acid improved silage fermentation. Waldo et al. (1971) reported higher energy

recoveries from unwilted silage treated with formic acid compared to no treatment.

Waldo et al. (1973) found that dairy heifers grew better on well made wilted silages than on direct cut silages treated with formic acid. In contrast, Castle and Watson (1970) found wilted silage was inferior to formic-treated silage for maintaining milk production.

Effectiveness of Organic Acids in Preventing Fungal Growth

Studies with Grains

Porter (1946) found organic acids had a greater inhibitory or germicidal effect than mineral acids at the same pH which was attributable to the whole (undissociated) molecule rather than solely to the hydrogen ion concentration. Weise (1971) tested the effect of adding 0.1, 0.3, 0.5, 1.0, 3.0, and 5% propionic acid to agar media on the growth of Candida, Pichia, Hansenula, Torulopsis, and mold. All but Torulopsis (which required .5%) were completely inhibited by .3% propionate. It was also noted that molding of cultures could be prevented by adding .25% Na propionate, but the inhibition of molds was greater than in yeasts.

Richardson and Halick (1957) showed that propionic acid or propionic anhydride inhibited the growth of molds and heating in

corn meal at 0.1% addition. Calcium propionate was effective at 0.3%, but no inhibition was shown for sodium propionate at .6%, or for propionamide or propionanalide at 0.3%. An acid environment was required for maximum effectiveness of propionate. Butyric, valeric, and caproic acids delayed heating when added at .1 to .2 percent and heat inhibiting activity decreased as chain length increased. However, prevention of mold appeared to increase with chain length. Sorbic acid also appeared very effective in depressing heat production.

Despite the decreases in spoilage due to acid addition, the authors (Richardson and Halick, 1957) concluded that drying was the only practical way to properly preserve feeds.

Sprague and Breniman (1969) reconstituted dry cracked corn to 20, 25, and 30% moisture and ensiled in quart jars. They concluded that the minimum moisture to provent mold in HMC was 30-33%. Drysdale (1970) estimated losses due to production of CO_2 during fermentation in sealed storage were as high as 5%. He suggested that the value of feed lost was almost equal to the cost of adding propionic acid which prevented CO_2 losses. The acid-preserved grain was found to keep for a year or more under open storage conditions.

Miller (1971) reported the amount of propionic acid needed for preservation was directly proportional to the moisture content of the grain. For HMC at 25% moisture, 1% propionic acid was sufficient while at 30% moisture 1.25% was necessary.

Jones et al. (1970) detected no heating or mold growth in HMC corn treated with three different mixtures of acetic, propionic, and butyric acid after 7 days of storage in sealed bags; however, in 7 days the controls molded (185,000-62,000 calories/g) had an off odor and heated. A pH of 4.4, which was obtained with 1% acid addition to 72% DM ear corn, was effective in inhibiting mold formation during 6 months of storage. However, Jones (1971) reported HM ear corn molded and heated within several weeks after treatment with 1.2% propionic acid.

Arends et al. (1972) treated 27% moisture corn with 1.5% acetic and propionic acids (60:40). Control corn was dried to 12% moisture. Mold spore counts of the untreated dried corn were 4.6 X 10^5/gm while the counts of the treated dried and treated HMC were 6.04 X 10^2 and 23, respectively. Christensen (1973) showed that treatment of sorghum (16-17% moisture) with 0.2, 0.4, or 0.8% propionic acid kept it free of molds for 483 days when held at 12 C. Sorghum at 19% moisture which was treated with .1 and .2% propionic at 27 C became heavily molded in 16 days. Corn (19-20% moisture) treated with .5% propionic and stored at 25 C was free of fungi after 54 days while samples at 30% moisture treated with 1.0% propionic plus acetic acids (60:40) were free of molds after 140 days when held at 20 C. Samples treated with enough acid to prevent molding had zero germination. Alfalfa pellets (16% moisture)

treated with .5% and .8% propionic acid had no molds after 210 days at 20 C. Other reports of prevention of molding in feeds stored for animals are by Jones et al. (1970), Marion et al. (1972), and Young et al. (1970).

Singh-Verma (1971) found propionic was more effective and longer lasting if added after deterioration of grain had already started because cells were growing in their log phase. Propionic acid was highly biocidal to saprophytic cocci, coliform bacteria, Aspergillus, Mucor, Rhizopus, Alternaria, and a variety of yeast. The growth of Bacillaceae, Actinomyces, Penicillium, Fusarium, and Cladosporium was also strongly inhibited. In contrast, Sauer et al. (1972) found higher levels of propionic or acetic were required when fungal invasion had already started. Fungi which grew in grain treated with insufficient amounts were the same type as in untreated grain.

Studies with Forages

Refermentation (spoilage upon removal from the silo) is often a serious problem to cattle feeders. With a clover-grass mixture Daniel et al. (1970) showed that propionic treatment reduced the frequency and intensity of refermentation. Hillman and Thomas (1973) found the addition of .4 to .6% propionic acid reduced molding of haylage in small silos when ensiled at 40% moisture.

Sleiman et al. (1972) treated whole chopped corn with various mixtures of formic, acetic, and propionic acids. All acids reduced temperature increases in silos. Days until molding were highest for propionic and lowest for control forages. Dry matter discarded was highest for acetic and lowest for acetic plus propionic treatments. All forages were readily consumed by growing heifers. It was concluded that the most effective retardation of spoilage was observed from adding propionic at 1% or higher.

Asplund (1971) reported that a mold inhibitor added to hay baled at 30% moisture prevented poor digestibility and acceptability usually associated with mold growth. Sleiman (1972) concluded that propionic acid could be used to prevent extreme losses of forages stored under minimal protection.

Henderson et al. (1972) found yeasts were more active in formic acid treated herbages than in untreated material. Losses due to fermentation plus oxidation in wilted silages were higher in the acid-treated than untreated silage. Total microbial counts were lower on formic acid treatment. Taylor and Phillips (1970) also found that total aerobic counts were lower, while lactate fermenters and thermophilic counts were higher in formic acid treated than untreated silages.

Non-Protein-Nitrogen Treatment of
Forages and Grains

Effects of Non-Protein-Nitrogen
on Fermentation and Animal

Schmutz et al. (1962) ensiled HMC at 24, 32, and 45% moisture. Lactobacilli and anaerobes were 10^9 per gm after 10 days for all treatments and yeasts numbered 10^9 for the 24% and 10^8 for the 45% corn at 60 days. Acetic acid was highest at 45% moisture. Lactic acid concentrations of the HMC were directly related to moisture content as were weight gains in growing heifers. When urea was added to the HMC 50% was degraded to ammonia by 20 days. Schmutz et al. (1964) noted that high moisture ear corn with added urea was initially lower in pH than untreated corn but this reversed during fermentation. Lactic acid was increased and acetic decreased with the addition of 1% monobasic calcium phosphate to HMC (Schmutz, et al. 1964).

Dutton and Otterby (1971) found that HMC treated with diammonium phosphate had higher pH values, NH_3 levels and greater nitrogen losses than controls or that treated with soybean meal; whereas the urea treatment was highest in acetic and lowest in propionic acid.

Huber and Santana (1972) found that dairy heifers ate more ammoniated than control silage when these were the only feeds offered. Also, lactating cows produced more milk on ammonia or untreated silages than on a negative control ration but no differences between the

non-protein-nitrogen treatments were noted. Ammoniated silage had higher lactic acid and water insoluble nitrogen than urea or control silages.

Webb et al. (1972) reported intraruminally-administered ammonium acetate gave greater rumen ammonia concentrations than did isonitrogenous quantities of urea. Urea elevated rumen pH but ammonium acetate did not. Both NPN supplements increased concentrations of ammonia and urea in peripheral blood. To produce toxicity it took less nitrogen from urea than ammonium acetate.

Allen et al. (1972) found no difference in gains of fattening steers supplemented with soybean meal, liquid urea, 1/2 liquid urea and 1/2 corn steep water, ammonium (NH_4) formate, NH_4 acetate, NH_4 propionate, NH_4 lactate, or NH_4 butyrate. Dutrow and Huber (1973) used urea, ammonium propionate, ammonium lactate or soybean meal to supply 80% or more of the supplemental nitrogen in high corn-corn silage rations for dairy cows and showed that fat corrected milk was significantly higher for the NPN than soybean meal diets. Dry matter intakes were not different between treatments.

Effect of Non-Protein-Nitrogen on Fungal Growth

Tomkins and Trout (1931) found green rot of citrus due to Penicillium digitatum was greatly reduced by storage in an atmosphere

of 500 to 1000 ppm ammonia. This concentration was produced by damp crystals of ammonium bicarbonate while the release of ammonia from ammonium acetate was not sufficient to prevent the rot. Tomkins (1932) reported that ammonia increased the latent period of fungal spore germination and that the same concentrations were needed to inhibit germination as growth.

Altschul et al. (1946) treated cottonseed with ammonia to inhibit plant respiration of mature seeds. They concluded that most of the deterioration which occurs in stored cottonseed is due to the action of enzymes in the seeds rather than microbial activity.

Bothast et al. (1973) treated 26 or 12% moisture corn with ammonia at 2 or 0.5% of the dry weight. Both concentrations of ammonia eliminated external and infecting molds and yeasts, and tended to reduce bacterial counts. Molds killed were species of Aspergillus, Penicillium, Fusarium, Trichoderma, and Rhizopus. Fusarium comprised 75% of external mold population while the rest were Aspergillus and Penicillium. Original corn had 7.9×10^4 molds and yeasts per gm, but after 24 hr. of tempering these increased to 5.1×10^5 per gm. Mice showed no preference for the tempered over the 2% ammoniated corn.

Conclusion

After reviewing the literature it becomes apparent that the growth of fungi in feeds during storage is a dangerous and expensive problem. Addition of propionic acid has been shown effective in retarding spoilage of high moisture grain. The effect of acid on fungal growth of forages has not been studied. Ammonia treatment of grains might also offer a less expensive preservative than organic acids, but further research on levels, animal acceptance, and methods of application is needed.

Little work has been done on patterns of fungal growth during referementation and the effectiveness of organic acids or ammonia in preventing losses during this period. Therefore, the objectives of this thesis are:

a) to evaluate the types of fungi during storage and refermentation of corn silage and HMC;

b) to determine the effects of short chain organic acid and ammonia additions to corn and corn silage on fungal development during storage and refermentation;

c) to ascertain the response of lactating cows to ammoniated corn grain.

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III.

PART A: FUNGAL GROWTH DURING FERMENTATION AND REFERMENTATION OF ORGANIC ACID TREATED CORN SILAGE

Abstract

Chopped corn (35% DM) in lots of 56 kg was treated with either propionic (P), formic (F), propionic + formic (PF), or propionic + acetic (PA) acids at either 0, 0.5, 1, or 2%. Each treatment and level was duplicated. Treated material was placed in polyethylene bags inside 200 ℓ drums and evacuated. During fermentation forages were sampled and temperatures measured at days 0, 3, 5, 15, 20, and 40. On day 40 when fermentation was complete, 12 kg portions of the silages were placed in open containers at 25 C. Samples were taken temperatures measured, and silages were aerated at days 0, 2, 14, 22, 29, and 36 to determine the effects of organic acids on refermentation of corn silage. Samples were analyzed for VFA, lactic acid, pH, and number and type of fungi.

Acid treatment at the 2% level reduced the average temperature when compared to controls (19.8 vs 24.4; $P < .01$) and .5 and 2% acid reduced ($P < .01$) the maximum temperature compared to controls during refermentation (33.6, 29.6, and 37.5, respectively). Propionic acid was more effective than other acids at .5 or 1% treatment. Lactic acid production was significantly depressed by 2% acid. All treatments containing propionic acid required more days ($P < .01$)

before visual molding (7.0, 13.4, 20.0, and 20.5 for F, PF, PA, and P, respectively). Days until complete spoilage was increased by acid treatment. All acids significantly decreased fungal colonies within 2 days after addition. During refermentation all treatments at the 1% level or lower exhibited a rapid increase in number of colonies; however, P showed a slower increase. The proportion of yeast was greatest at initiation of fermentation and decreased at day 40. During refermentation yeast started growth again. Geotrichum increased at day 40 of fermentation but was constant at other times. Aspergillus was significantly higher at day 40 of fermentation and 36 of refermentation. No significant amounts of Penicillium were detected at any date.

Introduction

Because of recent advances in synthesis of organic acids they have become economically available for use in preserving forages by preventing oxidation losses and fungal growth. Castle and Watson (1970) treated timothy and perennial rye grasses with .2% formic acid which had about a 7-15 C lower temperature increase than the untreated. Weise (1971) found that .3% propionic acid added to agar media retarded the growth of yeats and molds. Richardson and Halick (1957) inhibited the growth of molds and heating in corn meal with

propionic acid. Yeasts were reported higher in formic acid treated herbages (Henderson et al. 1972). Daniel et al. (1970) reported a reduction in frequency and intensity of after fermentation when a clover-grass mixture was treated with propionic acid. Sleiman et al. (1972) reported propionic acid at 1% was effective in retardation of spoilage in chopped corn. Sleiman (1972) also concluded that propionic acid could be used to prevent extreme losses of forages stored under minimal protection.

Fungi in forages have also been reported to cause tetany in milk cows (Lynch et al. 1969). Still et al. (1971) isolated Aspergillus ochraceus from molding hay that caused abortions in dairy cattle. Albright et al. (1964) diagnosed a gross hemorrhagic syndrome in 20 of 29 heifers fed a ration containing toxin-producing fungi and Mohanty et al. (1969) fed molded alfalfa hay to ruminant animals and found reduced DM intake, body weight gains, total VFA, ruminal ammonia, and total rumen protozoa.

The object of this experiment was to determine fungal growth and types of fungi during fermentation and refermentation in chopped corn forage after treating with different levels and combinations of organic acids. Changes in acid production, temperature, pH and dry matter in silages were also ascertained.

Materials and Methods

Whole corn plants (35% dry matter) were field chopped and brought to a storage area where 56 kg portions were treated with propionic acid (P), formic acid (F), acetic plus propionic acid (PA), and formic plus propionic acid (PF) acids at 0, 0.5%, 1% or 2% of the wet weight.[a] The PA acids mixture contained 80% propionic and 20% acetic acid while the PF treatment was 60% propionic and 40% formic acid. For treatment, the correct amount of chopped corn was weighed on a polyethylene sheet, and the acid was sprinkled on the surface while mixing with shovels. The entire mass was then rotated several times inside the polyethylene sheet to insure complete mixing.

Treated material was then sampled and transferred to two 200 ℓ metal drums, lined with polyethylene bags (5 mils thickness). The bags were then evacuated using a vacuum cleaner, sealed, and stored (drums with bags inside) in an enclosed barn. Additional samples were collected on days 1, 5, 10, 15, and 40 by opening the bags, quickly removing .5 kg portions, re-evacuating and sealing. Samples were immediately frozen and stored at -20 C. While the bags were open temperatures were measured with mercury thermometers inserted into the center of the silage mass.

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[a]Acids supplied by Celanese Chemical Co., Corpus Christi, TX.

At day 40 of fermentation the barrels were reopened and 12 kg portions were placed in open polyethylene containers and stored at 25 C. The remaining ensilage was weighed and discarded. Exposed silages were aerated by transferring from one container to another on days 1, 2, 3, 5, 7, 10, 14, 22, and 36 and sampled on days 2, 14, 22, 29, and 36. Aeration was done to maximize the re-fermentation rates of silages.

After thawing at room temperatures, forages were analyzed for DM, pH, lactic acid, and number and type of fungi. Dry matter (in duplicate) was determined by placing approximately 50 g wet forage in a forced-air oven at 90-100 C for 24 hours. Silages were prepared for pH, lactic, VFA, and fungal analyses by homogenizing 40 g in a Sorvall Omni-mixer.[a] The homogenizer cup was immersed in ice. Approximately 50 ml of the homogenized material was divided into two aliquots, one for pH[b] determination and the other placed in a container for plating to estimate fungal populations. Extracts from the remaining material were strained through two layers of cheesecloth and deproteinized with 50% sulfosalicylic acid (1 part SSA to 10 parts extract). The deproteinized extract was then centrifuged at 15,000 rpm for 10 minutes and the supernatant was stored in a freezer until analyzed for lactic acid and VFA.

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[a]Ivan Sorvall Inc., Newton, Conn.

[b]E. H. Sargent and Co., Chicago, Ill.

Colorimetric procedures of Barker and Summerson (1941) were used to determine lactic acid and VFAs were analyzed by injecting 3 µℓ of the deproteinized sample into a Hewlett Packard F & M gas chromatograph[a] using a glass column packed with chromasorb 101 (80/100 mesh).[b] The injection-port temperature was set at 340 C, the column at 285 C, and the flame detector at 320 C. Nitrogen was used as the carrier gas and flow rate was 30-40 mls per minute which created a retention time of approximately 7 minutes per sample. Sample VFA concentrations were calculated by comparing peak heights with a standard solution made with known weights of analytical grade acids in a stock solution and diluted until concentrations comparable with samples were reached.

Fungal population was determined by transferring with a sterile wide-mouth 10 ml pipette, 1 ml aliquots of the freshly homogenized silage sample into a dilution bottle filled with 99mls of sterile, distilled water. The sample was thoroughly mixed and serially diluted until the proper concentrations of fungal spores and mycelia were reached (approximately 20-50 colonies per plate). Either 1 or .1 ml of the diluted sample was then dispensed into sterile plastic petri dishes. Enough potato dextrose agar[c] with 100 mg per liter of novobiocin which had been melted and cooled to 45 C, was then added

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[a]Hewlett Packard F & M Scientific Co., Mocel 402.

[b]Johns-Manville, Celite Div., Denver, Colorado.

[c]BBL, Cockeysville, Maryland.

to cover the bottom of the petri dish and swirled to insure complete mixing of the agar and silage homogenate. After cooling, the plates were sealed and placed in the dark at 20 C for 7-10 days at which time the plates were removed and colonies were counted using a colony counter[a] and identified according to genus.

Results and Discussion

Heating of treated and control silages was negligible during fermentation and silage temperatures were usually within 1 to 2 degrees of ambient. One explanation for the lack of temperatures might be the anaerobic storage conditions after evacuation. However, due to the small mass of silage (56 kg) and the dramatic change in ambient temperatures (20 C on day 1 to 7.6 C on day 20) small changes in silage temperature might have been dissipated or overshadowed. Honig (1969) found DM losses increased linearly with the amount of added air and Federson (1971) reported oxidation was accompanied by large rises in temperature, but temperature increases in silos without oxygen present were negligible. Zimmer and Gordon (1964) found aeration increased CO_2 and DM losses. Complete anaerobiosis was apparently

[a]Fisher Scientific Co., New York, N.Y.

achieved in this experiment as indicated by the negligible DM losses which analyzed less than 3% on control silages.

Refermentation is very critical during feeding of a stored feed such as haylage, HMC, or silage. In addition to the loss of nutrients due to refermentation (aerobic fermentation on removal from the silo) as discussed by Daniel et al. (1970), toxic fungal growth may also occur. Losses or damage of a feed from the time of exposure to air until its consumption are not generally known but they are largely dependent on environmental conditions. For these reasons the influence of organic acids on refermentation was evaluated. Because fungal growth is closely associated with heating of stored feeds (Gilman and Barron, 1930; Milner and Geddes, 1946; Milner and Geddes, 1945; and Federson, 1971) measurements of both parameters were measured.

Aeration caused a marked increase in temperatures as shown earlier by Federson (1971). Average temperatures (Table 1) during refermentation were depressed at all levels of addition for all acids,

TABLE 1.--Effect of Level of Organic Acid Addition on Temperature Development in Corn Silage During Refermentation

LEVEL OF ACID %	TEMPERATURE (AVERAGE C)	MAXIMUM TEMPERATURE (C)
0%	24.4^{A}	37.5^{A}
0.5%	20.8^{AB}	33.6^{C}
1.0%	20.2^{AB}	35.7^{AC}
2.0%	19.8^{B}	29.6^{B}

^{ABC}Means not sharing same superscript are different ($P < .01$).

but significantly so ($P < .01$) at 2.0%. Maximum temperatures during refermentation were also less for all acid additions, but the decrease was significant ($P < .01$) at the 0.5 and 2.0% levels. This agrees with the work of Daniel et al. (1970) who found propionic acid treatment reduced the frequency and intensity of after-fermentation. Richardson and Halick (1957) also found propionic acid inhibited heating in corn meal and Sleiman (1972) reported formic or acetic plus propionic acids reduced temperatures of unprotected rye forage.

No significant differences were found between acid treatments with respect to heating. However, all but one series of treatments contained propionic acid and that series was treated with formic acid. Castle and Watson (1970) found formic acid-treated silages had a 7-15 C lower temperatures during fermentation than controls. It is difficult to explain why the .5% level of acids significantly reduced maximum temperatures and 1% did not.

Lactic acid concentrations (across all dates and treatment levels) in silages (Table 2) were significantly decreased ($P < .01$) by 2% addition of all acids, (2.54, 1.79, 1.56, and .65% of DM for 0, .5, 1, and 2% acid additions, respectively). This indicates a severe inhibition of microbial activity by acids during both fermentation and refermentation. However, of more interest would be the peak level of lactic acid which would be similar to silage which would be fed from the silo. Lactic acid concentrations were

TABLE 2.--Lactic Acid Production (% of DM)[B] in Organic Acid Treated Corn Silage During Fermentation and Refermentation.

	Days of Fermentation					Days of Refermentation					
	0	3	5	15	20	40:0	2	14	22	29	36
Control	0	1.86	7.18	7.93	5.75	5.92	5.90	2.13	0	A	A
.5% P	0	0	1.23	4.24	4.71	4.49	4.77	0.11	0.29	1.34	4.47
1.0% P	0	0	0.17	0	0	0.63	0.32	4.43	2.11	2.26	0.88
2.0% P[C]	0	0	0.22	0	0	0.10	0.06	0	0.13	0	0.33
.5% F	0	0	0.19	0.09	0.40	0.23	0.55	2.54	1.60	1.20	2.37
1.0% F	0.1	0	0	0	0.04	0	0	4.14	1.27	2.69	2.04
2.0% F[C]	0	0	0	0	0.04	0	0	4.90	2.53	0	A
.5% P+F	0	0	0.14	1.11	1.09	1.62	2.74	2.55	1.41	0.10	6.64
1.0% P+F	0	0	0	0	0	0	0.10	5.64	1.53	0.77	2.48
2.0% P+F[C]	0.1	0	0	0	0	0.06	0.20	0.25	1.82	1.99	0.83
.5% P+A	0.1	0	2.71	5.42	4.41	3.70	5.82	3.51	1.71	1.87	1.82
1.0% P+A	0.1	0	0.07	0	1.64	0.97	3.83	4.60	1.10	.50	0
2.0% P+A	0.2	0	0	0	0	0.07	0.20	0.15	0.19	.62	1.92

[A]Missing values indicate complete spoilage of silage.

[B]Each value is the average of two duplicates.

[C]2% acid addition reduced the production of lactic acid compared to no treatment (2.54 vs .65 $P < .01$).

apparently maximal after 15 days of fermentation which agrees with Langston et al. (1958).

Lactic acid on days 20 and 40 of fermentation for the control silage was 5.8% of the dry matter (Table 2 and Figure 1). At .5% propionic or propionic plus acetic treatment, lactate was depressed to 4.0% or about 70% of the controls. Strong inhibition of lactic acid production was observed on both .5% treatments containing formic acid. This different affect of the acids when applied at the same rate may be due to the relative strengths of the acids (Carpintero et al. 1969). The pH (Table 3) reflect this difference with the .5% propionic and propionic plus acetic acids having pH's of 4.80 and 4.73 while the two formic acid treatments averaged 4.47. This finding is in general agreement with Huber et al. (1972) who observed marked decreases in lactic acid of normal corn silage treated with formic acid at .3 or .6%, but smaller decreases with added acetic or propionic acids.

All acid treatments at the 2% level completely inhibited lactic acid production during fermentation due to the low pH's caused by treatments (Table 2 and Figure 2). The pH's were 3.28 and 3.65 for the formic and formic plus acetic and 4.20 and 4.23 for the propionic and propionic plus acetic.

During refermentation lactic acid decreased markedly on the control, .5% propionic and .5% propionic plus acetic while a smaller

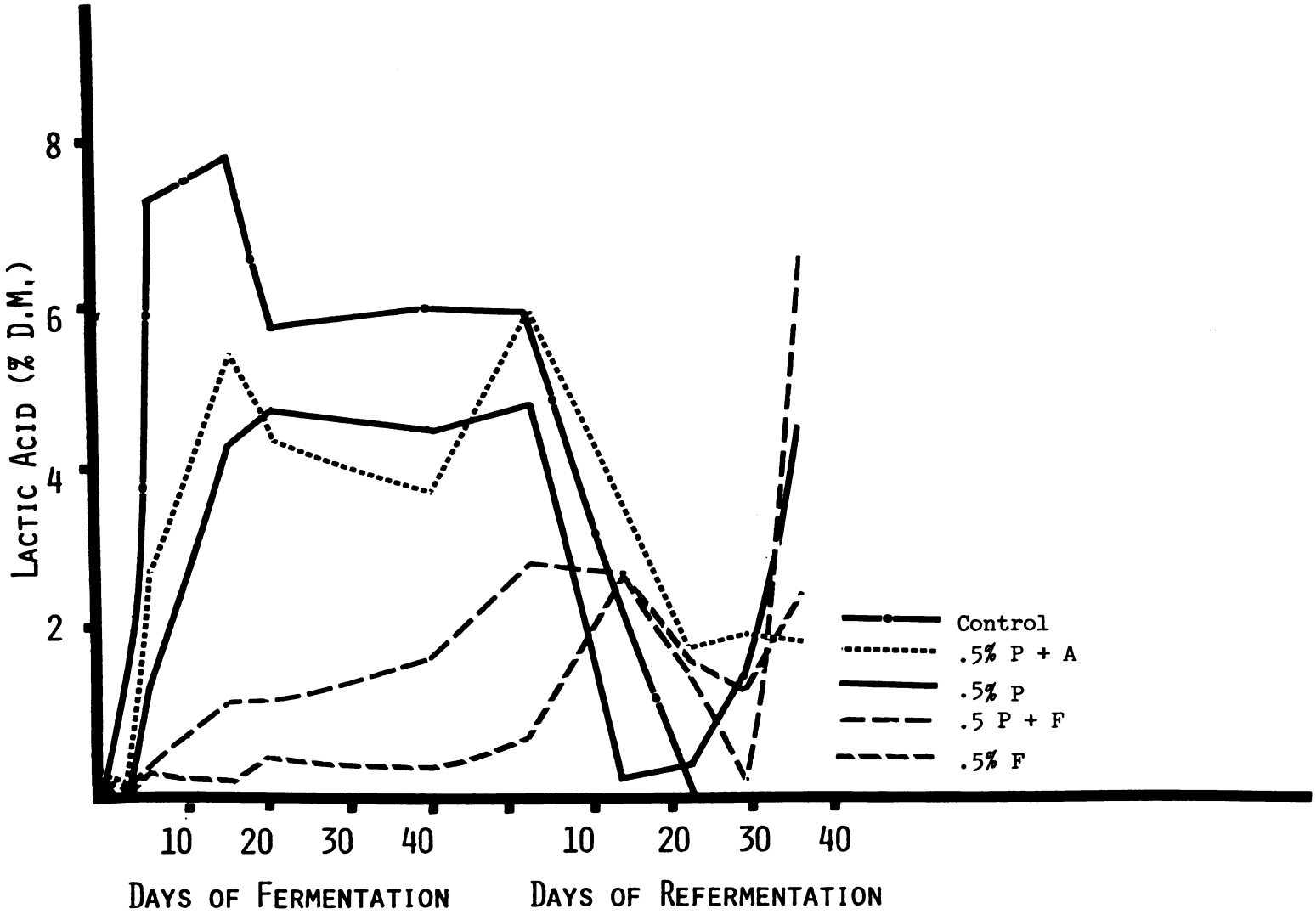

Fig. 1.--Lactic Acid in Control and 0.5% Acid Treated Corn Silage

TABLE 3.--PH s[B] of Organic Acid Treated Corn Silage During Fermentation and Refermentation.

	Days of Fermentation					Days of Refermentation					
	0	3	5	15	20	40:0	2	14	22	29	36
Control	5.90	4.35	4.25	4.03	4.15	3.93	4.08	5.80	7.95	A	A
.5% P	4.80	4.65	4.53	4.07	4.25	4.05	3.95	5.20	5.95	4.90	5.05
1.0% P	4.50	4.40	4.38	4.38	4.20	4.53	4.38	3.93	4.25	4.64	4.64
2.0% P	4.20	4.15	4.23	4.14	4.23	4.23	4.28	4.18	4.18	4.20	4.20
.5% F	4.30	4.02	4.35	4.30	4.50	4.38	4.35	4.18	4.30	4.48	5.30
1.0% F	3.73	3.65	3.70	3.69	3.68	4.45	4.85	4.30	5.90	4.60	4.65
2.0% F	3.28	3.18	3.18	3.21	3.35	4.35	4.70	4.15	5.05	7.05	A
.5% P+F	4.65	4.55	4.55	4.35	4.40	4.43	4.20	4.05	4.73	5.05	4.70
1.0% P+F	4.15	4.10	4.10	4.06	4.13	4.38	4.28	4.35	5.05	5.29	5.06
2.0% P+F	3.65	3.55	3.56	4.55	3.83	3.60	3.58	4.13	4.15	4.14	4.40
.5% P+A	4.73	4.55	4.33	3.95	3.95	3.95	3.90	4.15	4.50	5.43	5.10
1.0% P+A	4.48	4.38	4.43	4.28	4.43	4.30	4.15	5.15	5.00	5.05	5.25
2.0% P+A	4.23	4.15	4.18	4.13	3.99	4.33	4.20	4.23	4.34	4.30	4.23

[A]Missing values indicate complete spoilage of silage.

[B]Each value is the average of two duplicates.

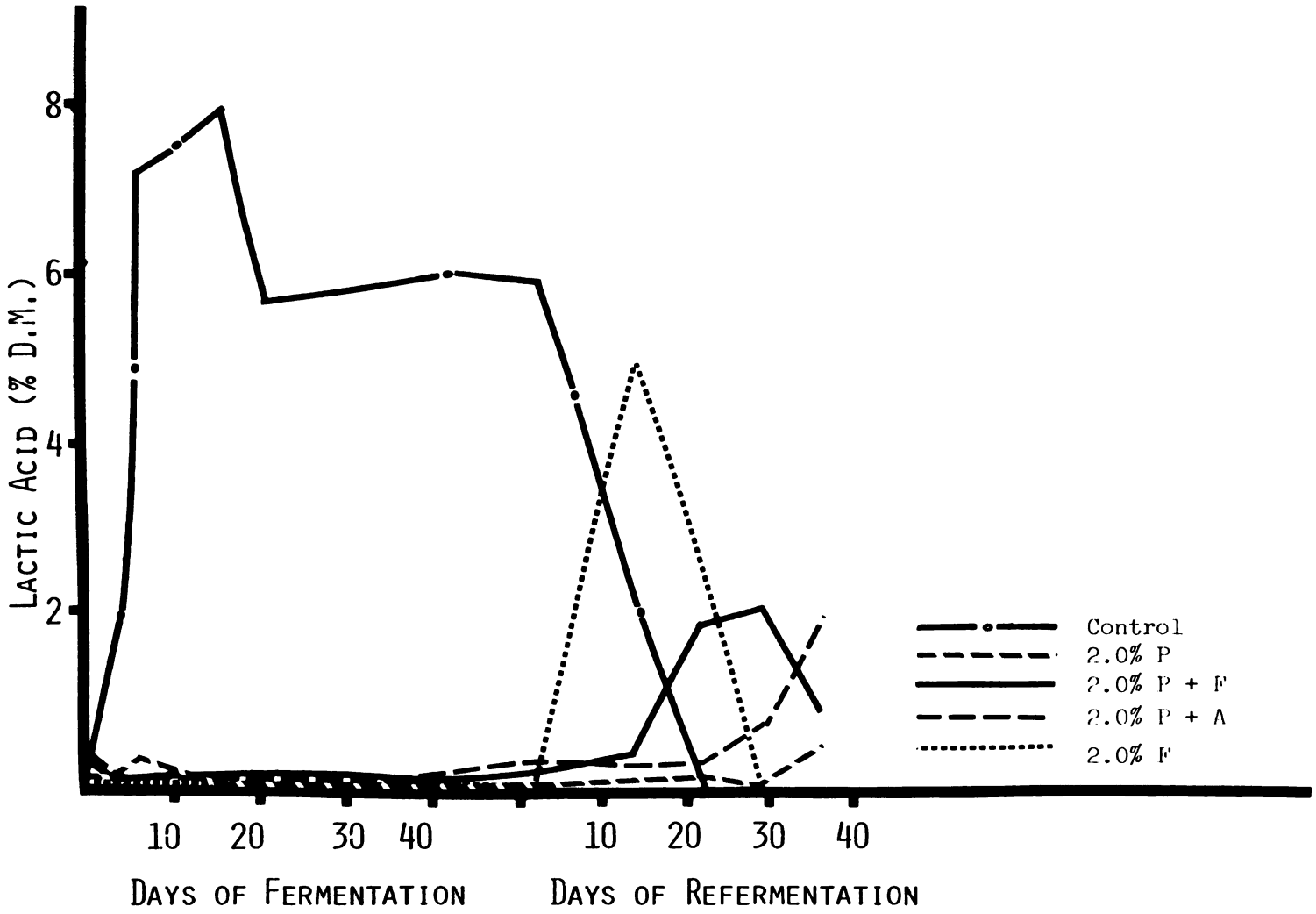

Fig. 2.--Lactic Acid in Control and 2% Acid Treated Corn Silage

decrease was observed on the formic treatments (Figure 1). After 30 days of refermentation a marked increase of lactic acid occurred on the .5% propionic, .5% formic, and .5% propionic plus formic. On the 2% formic treatments sharp increases in lactic acid were noted at the beginning of refermentation (Figure 2). These increases preceded those on the propionic treatments by about 3 weeks. The formic acid was being removed from the treated material faster than the propionic acid which permitted an earlier lactate production. This is substantiated by the rapid increase in pH to 4.70 at 1% formic treatment on day 2 of refermentation while the pH of the 2% propionic remained at 4.28 (Table 3).

On treatments showing low lactate levels during fermentation, the pH and lactate began to increase considerably during refermentation. The water soluble carbohydrates were preserved by the acid treatments, but once the pH increased, lactate-producing bacteria began to proliferate. Apparently the lactate was metabolized during later stages of refermentation (Table 2). A similar pattern was reported by Sleiman (1972) who found that after complete spoilage lactic acid was not found in control or acid-treated silages.

Acetic acid production follows the pattern of lactic acid. Production at day 20 was decreased by .5% formic and formic plus propionic treatments and was inhibited by all treatments at 1% or greater addition except the treatment which contained acetic acid (propionic +

acetic). Although fermentation was inhibited by 2% added acid as indicated by lactic acid (Figure 2) the added acetic acid increased the measured level comparable with control. During refermentation, acetic acid increased most in silages which had not been fermented, and at spoilage a general decline was noted. (Table 4)

Propionate (Table 5) and pH (Table 3) were not statistically analyzed because the large acid additions exerted strong effects on both parameters. The pH data (Table 3) substantiate those of Carpintero et al. (1969) who reported the strongly acidic nature of formic acid which in this experiment, decreased pH to 3.28 at 2% addition compared to 4.20 for 2% propionic addition. Propionic acid production (Table 5) was low on all treatments and increases on the propionate treatments reflect that propionate added, which agrees with Sleiman (1972).

Correlations between propionate, pH, temperature, and spoilage were determined (Table 6). Average and maximum temperatures were positively correlated ($P < .01$) with pH. Levels of treatment and maximum temperatures were negatively correlated ($P < .01$). Days until visual fungi and days until spoilage were negatively correlated with pH ($P < .01$). In addition a high positive correlation of .836 ($P < .01$) was found between propionate and days until spoilage. Hence, the pH increases during refermentation were accompanied by spoilage; but whether this change in pH is the cause or result of fungal growth

TABLE 4.--Acetic Acid Content[A] (% DM) of Organic Acid Treated Corn Silage During Fermentation and Refermentation

	FERMENTATION					REFERMENTATION			
	0	3	5	15	20	40:0	2	14	22
CONTROL	.39	.95	1.09	1.32	1.43	.84	2.51	1.45	1.16
.5% P	.35	.46	.42	.25	1.27	.89	3.44	1.07	2.08
.5% F	.37	.50	.20	.50	.49	.59	..56	4.20	3.35
.5% P+F	.35	.40	.22	.26	.54	.79	1.80	5.79	2.02
.5% P+A	.75	.82	.60	.88	1.36	1.97	3.40	3.81	2.45
1.0% P	.27	.48	.26	.26	.36	.68	1.03	.76	.70
1.0% F	.45	.65	.24	.26	.41	.57	1.23	3.97	4.13
1.0% P+F	.34	.50	.31	.38	.38	.48	1.69	2.42	2.58
1.0% P+A	1.14	1.21	.75	1.36	.52	.74	1.85	.79	.83
2.0% P	.27	.41	.41	.54	.48	.78	1.61	1.68	1.06
2.0% F	.48	1.23	.65	.36	.69	.86	1.72	4.21	1.17
2.0% P+F	.44	.62	.25	1.33	.40	.67	.97	2.62	.51
2.0% P+A	.98	1.23	1.08	1.47	1.19	1.89	1.15	.50	.31

[A]Each treatment is the mean of two duplicates.

TABLE 5.--Propionic Acid Content (% DM) of Organic Acid Treated Corn Silage During Fermentation and Refermentation

	FERMENTATION					REFERMENTATION			
	0	3	5	15	20	40:0	2	14	22
CONTROL	.27	.25	.23	.12	.43	.27	.35	.36	.30
.5% P	1.18	1.59	1.20	1.27	1.27	.58	2.10	3.26	3.10
1% P	2.37	3.57	2.82	2.63	1.84	1.84	3.70	3.02	4.10
2% P	4.09	3.03	4.84	4.14	4.12	3.38	4.71	5.78	7.48
.5% F	.40	.46	.36	.42	.29	.44	.36	.87	1.40
1.0% F	.20	2.05	.09	.38	.12	.28	.24	.44	.44
2.0% F	.09	.50	.25	.19	.38	.21	.21	.29	.23
.5% F+P	.80	.99	.58	.68	.60	.59	1.14	1.23	1.51
1% F+P	1.46	1.68	1.50	1.51	1.23	.91	2.61	1.32	.86
2% F+P	5.89	4.59	3.44	3.46	2.92	2.72	5.22	4.19	5.25
5% A+P	1.76	1.25	.91	1.07	.98	1.10	1.70	1.24	1.70
1.0% A+P	3.19	2.54	2.14	2.10	1.63	1.64	3.10	2.63	3.03
2.0% A+P	3.58	4.08	3.61	4.09	3.48	3.53	3.79	3.98	3.61

TABLE 6.--Correlations of Several Variables During Fermentation and Refermentation of Organic Acid Treated Corn Silage

VARIABLES	r	LEVEL OF SIGNIFICANCE
PH: Temperature	.530	.01
PH: Fungi	.701	.01
PH: Maximum Temperature	.717	.01
PH: Days until Visual Fungi	-.691	.01
PH: Days until Spoilage	-.583	.01
Propionate: Days until Spoilage	.836	.01
Level: Maximum Temperature	-.613	.01

is not known. The high correlation between days until spoilage and propionate confirms its action as an effective fungicide.

As the level of acid addition increased days until visual fungal growth and days until complete spoilage also increased ($P < .01$; Table 7). This agrees with Sleiman (1972) who found acid treatment delayed spoilage of corn silage. Propionic acid was more effective than formic acid in retarding spoilage. Addition of formic to propionic acid decreased the effectiveness of the propionic acid as a fungicide as shown by 13.4 vs 20.5 days until visual fungi appeared, but this was not observed with the propionic: acetic mixture. These

TABLE 7.--Number of Days until Fungi Were Noted on Corn Silage, Complete Spoilage and DM Loss During Refermentation as Affected by Level and Type of Acid

LEVEL OF ACID	DAYS UNTIL VISUAL FUNGAL GROWTH	DAYS UNTIL COMPLETE SPOILAGE
0%	5.0^{A}	18.0^{A}
.5%	7.5^{A}	29.8^{B}
1.0%	19.9^{B}	34.3^{B}
2.0%	28.5^{B}	32.5^{B}
ACID TREATMENT		
Propionic	20.5^{A}	28.8
Formic	7.0^{B}	26.3
Propionic + Formic	13.4^{C}	29.8
Propionic + Acetic	20.0^{A}	29.8

^{ABC}Means not sharing same superscript are different ($P < .01$).

data agree with Richardson and Halick (1957) who found propionic acid a very effective fungicide.

Addition of 2% acid reduced ($P < .01$) DM losses during fermentation compared to the three lower levels of acid addition. Losses (as % of the original DM) averaged 1.5, 1.5, 1.3, and .5 kg for 0, .5, 1, and 2% acid addition, respectively. Apparently, this was due to the

nearly complete inhibition of fermentation by all acids added at 2% of the treated silage.

The literature revealed no reference to a method for quantification of fungi in forages. A comparison of media with additives was used for the identification and enumeration of fungi using corn silage homogenized in distilled sterile water (Table 8). The media

TABLE 8.--Number of Fungal Colonies (per gm x 10^5) on Different Media, with or without Novobiocin and/or Rose Bengal

	MEDIA				
	PDA	MALT	CHRISTIANSEN'S MALT MEDIUM	CORN MEAL	$\bar{X}$
NO ADDITIVE	49	57	0	48	51
Rose Bengal[A]	23	22	0	14	19
Novobiocin[B]	19	16	0	18	17
Rose Bengal Novobiocin	16	17	0	20	18

[A] 33.3 mgs per liter of media.
[B] 100 mgs per liter of media.

compared were: potato dextrose agar (PDA) (formulated to identify fungi because of its low pH); malt agar; corn meal agar and Christensen's medium (Christensen, 1946). All tests were conducted with the following: 1) no additive, 2) rose bengal, 3) novobiocin, or 4)

rose bengal plus novobiocin. Rose bengal was used to retard the growth of Mucor sp. but after determining insignificant amounts of this contaminant present its use was discarded. Novobiocin was added to retard bacterial growth. Christensen's medium appeared unsatisfactory and slightly higher counts were obtained on the PDA than the corn meal and malt agar.

Corn silage contains a wide variety of fungi during fermentation and refermentation. PDA is used to identify many types of fungi. Because of this and slightly greater numbers of fungi isolated, PDA was chosen as the medium used in this study. Novobiocin was used because of its bactericidal properties. The apparent higher counts on the medium which did not contain novobiocin was because bacterial colonies were contaminating the fungal colonies and were being included in the fungal counts (Table 8). No differences in types of fungi isolated were seen between PDA, corn meal or malt extract. The same type of colonies were also observed when inoculum was sprinkled on solidified agar.

Total fungal colonies represents the approximate number of fungi present in the samples. By the methods used it was not possible to differentiate between colonies grown from mycelia or spores. It is quite possible that all spores or mycelia did not grow; however, for comparative purposes this procedure was satisfactory.

Fungal colonies (Table 9) in green chopped corn were significantly decreased ($P < .05$) within one hour after acid addition. The fungal population of all silages decreased during fermentation, which agrees with Federson (1971). Upon aeration (refermentation) fungal colonies increased significantly ($P < .01$) and continued to increase until day 36 of refermentation (Table 10 and Figure 3). Acid addition across all dates and treatments significantly reduced the number of fungal colonies obtained in plates (Table 11) which agrees with data of Sleiman (1972).

For acid treatment of silage to be economical it must be effective at an application rate of 1.0% or less. Fungal population in material treated at 1% (Figure 3) reached a low level during fermentation. With the exception of the propionic plus formic acid treatment, all material treated with propionic acid or a mixture of propionic had a marked lower rate of fungal growth during refermentation than formic or control. Propionic alone had a slower rate of fungal growth. On day 36 at the .5% treatment level (Table 9) propionic acid and propionic plus acetic appeared more effective than the two treatments containing formic acid. Formic acid alone was very ineffective as a fungicide even at the 2% level.

Propionic acid was present after 22 days of refermentation (Table 5) so the increases in fungi were due to an adaptation to propionic acid and not its disappearance. The formic disappeared at a

TABLE 9.--Total Fungal Colonies[A] (total counts/gm x 10^3) in Organic Acid Treated Corn Silage During Fermentation and Refermentation.

	Days of Fermentation					Days of Refermentation					
	0	3	5	15	20	40:0	2	14	22	29	36
Control	820	38	16	3	23	12	24	18,862	105,500	B	B
.5% P	88	39	5	4	1	6	0	838	525	3,575	1,465
1.0% P	18	18	16	10	4	2	1	6	21	91	405
2.0% P	10	21	17	6	10	15	21	21	28	7	48
.5% F	38	63	8	2	14	274	36	24	26	2,223	19,035
1.0% F	63	86	3	1	0	10	7	13	408	375	35
2.0% F	23	0	0	0	0	2	17	81	34,357	B	B
.5% P+F	18	22	9	2	78	28	8	5	1,920	27,100	10,000
1.0% P+F	38	130	28	2	18	39	5	54	3,855	1,461	259
2.0% P+F	12	20	47	0	0	3	1	14	54	43	98
.5% P+A	61	36	14	0	2	1	10	68	47	63,921	1,020
1.0% P+A	62	90	53	2	3	3	25	19	122	1,145	33
2.0% P+A	29	32	11	2	6	58	0	26	10	35	16

[A]Each value is the average of two duplicates.

[B]Missing values indicate complete spoilage of silage.

TABLE 10.--Number of Fungal Colonies Obtained from Organic Acid Treated Corn Silage as Affected by Date

DAY	NUMBER OF COLONIES (10^3/gm)
FERMENTATION	
1	234^A
40	31^A
REFERMENTATION	
14	$4,789^B$
36	$21,387^C$

^{ABC}Means not sharing same superscript are different ($P < .01$).

TABLE 11.--Number of Fungal Colonies as Affected by Level of Acid Addition

LEVEL OF ACID	NUMBER OF COLONIES (10^3/gm)
0%	$22,486^A$
0.5%	$3,286^B$
1.0%	384^B
2.0%	284^B

^{AB}Means not sharing same superscript are different ($P < .01$).

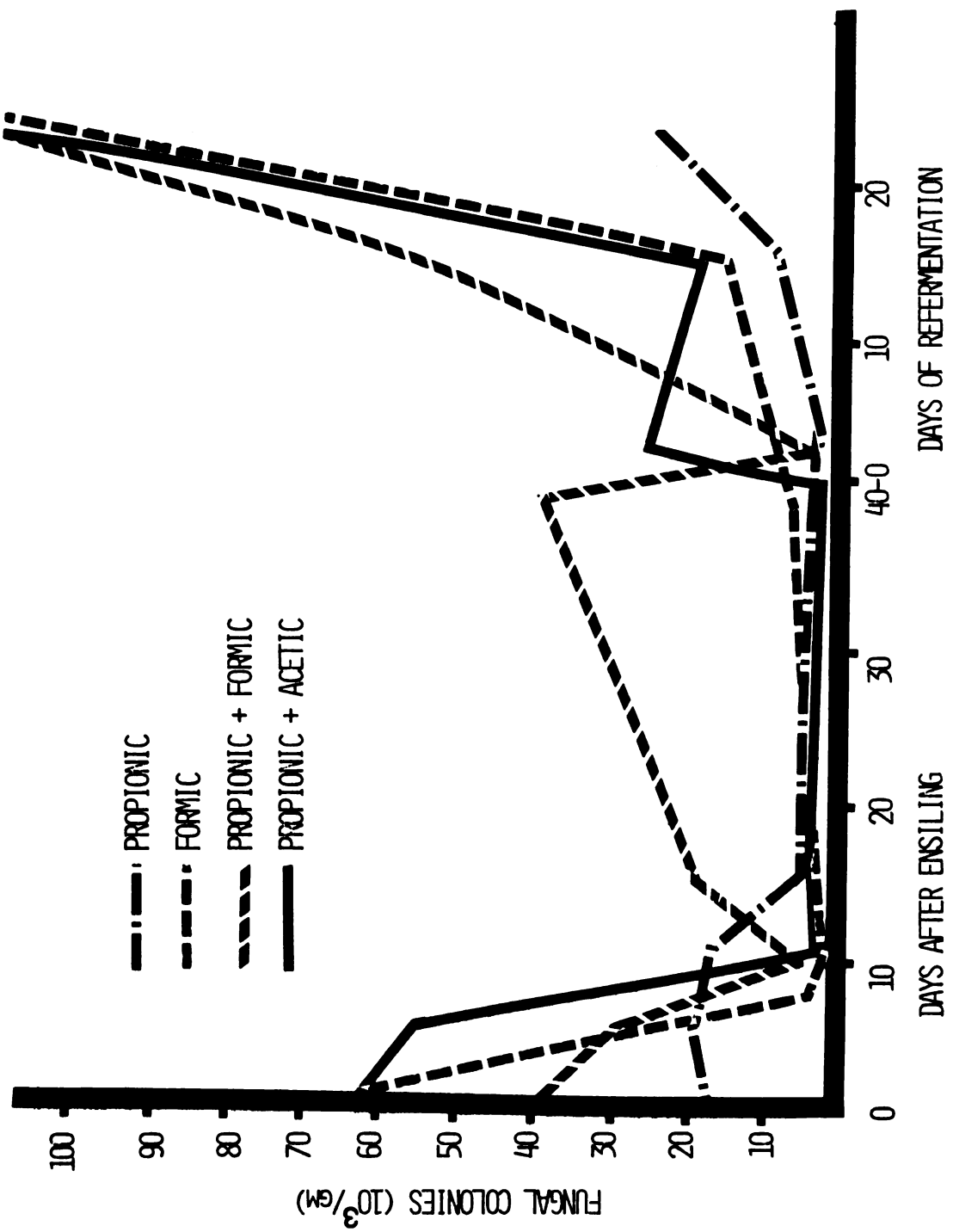

Fig. 3.--Fungal Growth in Corn Silage Treated with 1.0% Acid from 4 Sources

faster rate than the propionic as indicated by the pH data (Table 3) so the fungicidal effect of formic whether it be pH or the molecule itself is lost at a faster rate than propionic acid.

The proportion of yeast (Table 12) was highest at initiation of fermentation and decreased at day 40 while Geotrichium increased during fermentation. During refermentation yeast growth was again accelerated but depressed near the end of refermentation. Aspergillus was highest on day 40 of fermentation and 36 of refermentation. No significant amounts of Penicillium were detected at any date.

TABLE 12.--Types of Platable Fungal Colonies at Day 1 and 40 During Fermentation and Day 14 and 36 During Refermentation.

DAY		YEAST	GEOTRICHUM	ASPERGILLUS sp.	PENICILLUM sp.
1	FERMENTATION	71.7%^{C}	17.3%^{A}	0.1%^{A}	.04%
40	FERMENTATION	8.4%^{A}	35.2%^{B}	50.5%^{B}	.07%
14	REFERMENTATION	65.3%^{C}	26.7%^{AB}	7.2%^{A}	.00
36	REFERMENTATION	38.5%^{B}	16.4%^{A}	44.3%^{B}	.00

^{ABC}Means not sharing same superscript are different ($P < .01$).

No change in relative proportions of specific fungi resulted from the addition of organic acids. The primary effect of the acids appears to be to delay fungal growth. Yeast play an important role in the fermentation and refermentation of stored forages and are not

know to produce toxins during feed storage. Upon spoilage Aspergillus became dominant but was not affected by treatment so upon spoilage the same possibility exists of toxin contamination in acid treated silage as untreated but the time until spoilage is increased and nutrient loss decreased by the acid treatments (particularly propionic).

Summary and Conclusions

Organic acids can be used to decrease refermentation in high moisture feeds. Propionic appears to be much more effective than formic acid in feed preservation. The addition of formic acid to propionic acid decreased the effectiveness of propionic acid. Propionic acid significantly ($P < .01$) increased the number of days until spoilage. The addition of organic acids did not change the type of fungal flora present but delayed its growth. All levels of formic acid severely inhibited lactic acid production while the inhibition on the propionic treatment was small at the .5% treatment level and became severe at 2%. At the present cost of acid it appears that the maximum level of addition is approximately .5%. At this level propionic acid appears to be a suitable additive to decrease spoilage and refermentation.

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IV.

PART B: FUNGAL GROWTH DURING FERMENTATION AND REFERMENTATION OF NON-PROTEIN-NITROGEN TREATED CORN SILAGE

Abstract

Fifty-six kg portions of chopped whole corn plant (35% DM) were treated with either urea, aqua-ammonia, or ammonia-molasses solution at 0%, .2%, .4%, or .8% added nitrogen, placed in polyethylene bags inside 200 ℓ metal drums, evacuated, and sealed. During fermentation forages were sampled and temperature measured on days 0, 5, 20, and 86. On day 86 two 12 kg portions were placed in open containers at 25 C. On days 0, 7, 21, and 28 of refermentation silages were sampled, aerated and temperatures measured. Samples were analyzed for VFA, lactic acid, pH, and number and type of fungi. Initial pH increased with nitrogen addition. At .8% nitrogen pH values for urea, aqua-ammonia, and ammonia-solution were 6.35, 9.88, and 9.65 respectively. Added nitrogen also resulted in large pH increases during refermentation. Ammonia added at .2% nitrogen stimulated, but higher levels depressed lactate production in silages. Upon spoilage lactic acid disappeared. Silages treated with the ammonia-solution required the longest time to spoil ($P < .01$). Ammonia nitrogen reduced total fungal counts ($P < .01$) at 30 minutes after treatment but during fermentation no differences in fungal growth between treatments were noted. At both .4 and .8% nitrogen fungal counts were lower ($P < .01$)

than at 0 and .2%. Treatment did not significantly change the relative proportions of fungi during spoilage. The majority were yeast and Geotrichum, while some Aspergillus and Penicillium were identified. However, yeast decreased and Geotrichum increased during spoilage on all treatments ($P < .01$).

Introduction

Mohanty et al. (1969) fed moldy alfalfa hay to ruminants and found reduced dry matter intake, body weight gains, total VFA, ruminal ammonia, and total rumen protozoa. Albright et al. (1964) diagnosed a gross hemorragic syndrome in 20 of 29 heifers fed a ration containing toxin-producing fungi. Fungal growth has been reported to cause tetany in milk cows (Lynch et al. 1969); and Still et al. (1971) isolated Aspergillus ochraceus from moldy hay which they reported as the cause of abortions in dairy cattle.

Tomkins and Trout (1931) found green rot in citrus was largely reduced by storage in a dilute ammonia atmosphere which could be provided by ammonium bicarbonate but not ammonium acetate. Ammonia increased the latent period of fungal spore germination (Tomkins, 1932). Bothast et al. (1973) treated 12 or 26% moisture corn with ammonia at

2 or .5% of dry weight and reported that both concentrations eliminated external and infecting molds and yeasts.

Huber and Santana (1972) compared corn silage treated with an ammonia solution at ensiling time with urea or control silages. Heifers ate more ammonia silage than control and NPN supplemented rations supported more milk production than the negative control. Ammonia silage was higher in lactic acid, but there was no difference in animal performance between the urea and ammonia treatments. However, more recent experiments suggest a slight superiority of ammonia over urea.

The purpose of this study was to determine the effects of urea, aqua-ammonia and an ammonia-molasses-mineral mixture on fungal growth and types of fungi during ensiling and refermentation of chopped corn. In addition changes in fermentation acids, temperature, pH, and dry matter were determined.

Materials and Methods

Corn (approximately 35% dry matter) was field chopped and brought to the storage area where duplicate fifty-six kg lots were treated with 0, .2, .4, or .8% added nitrogen as urea, aqua-ammonia (aqua-NH_4; water plus anhydrous ammonia, or an NH_4-molasses, mineral

solution (NH_4-solution; trade name Prosil). This required the addition of .5, 1, or 2% urea; 1, 2, or 4% aqua-NH_4; or 1.8, 3.6, or 7.2% NH_4-solution. The weighed material was spread on a polyethylene sheet and sprinkled with the nitrogen compound. After blending with shovels the entire mass was rotated on the polyethylene sheet to insure complete mixing.

Treated material was then transferred to 200 ℓ metal drums lined with transparent polyethylene bags (5 mils in thickness). Samples (.5 kg) were removed and placed inside smaller bags and immediately frozen at -20 C. The polyethylene bags containing the treated material was then evacuated by the use of a vacuum cleaner and sealed. Containers were stored in an enclosed barn on a concrete floor. On days 0, 1, 5, 10, 15, and 86 bags were opened, .5 kg samples were removed quickly, and bags were re-evacuated and sealed. While the bags were open temperatures were determined with mercury thermometers inserted into the center of the silage mass.

After 86 days of fermentation, 12 kg portions of the silage were placed in open polyethylene containers and stored at 25 C. The remaining silage was weighed and discarded. The material was aerated by transferring from one container to another on days 1, 2, 3, 5, 7, 11, 14, 21, and 27 and samples (.5 kg) were removed on days 1, 7, 21, and 28.

Analyses of DM, pH, lactic acid, VFA, and fungi were performed according to the procedures described in Part A.

Results and Discussion

The pH values for the ammonia treated silages were higher than those for control or urea treatments 30 minutes after ensiling (Figure 4); but by 10 days pH of silages with .2% N as ammonia was similar to control and urea treatments. Upon refermentation, the pH of nitrogen treatments increased at a faster rate than controls. At the .8% N as ammonia the pH during refermentation never declined below 6.0 and it rapidly increased to 8.0 or more during refermentation (Table 1, Appendix).

The pH values observed for ammonia added at .4 and .8% N were above those for optimum growth of lactic acid bacteria (Langston et al. 1958); therefore decreased lactic acid was observed on these treatments (Figure 5). In fact, despite an abundance of water soluble carbohydrates lactic acid was almost completely inhibited at .8% ammonia N, while less inhibition occurred at the .4%. At the .2% ammonia N lactic acid was higher than controls or .2% urea N; whereas, a stimulation of lactic acid was observed at both .4 and .8% urea N. The pH of urea treated silages was never above 5 except early in

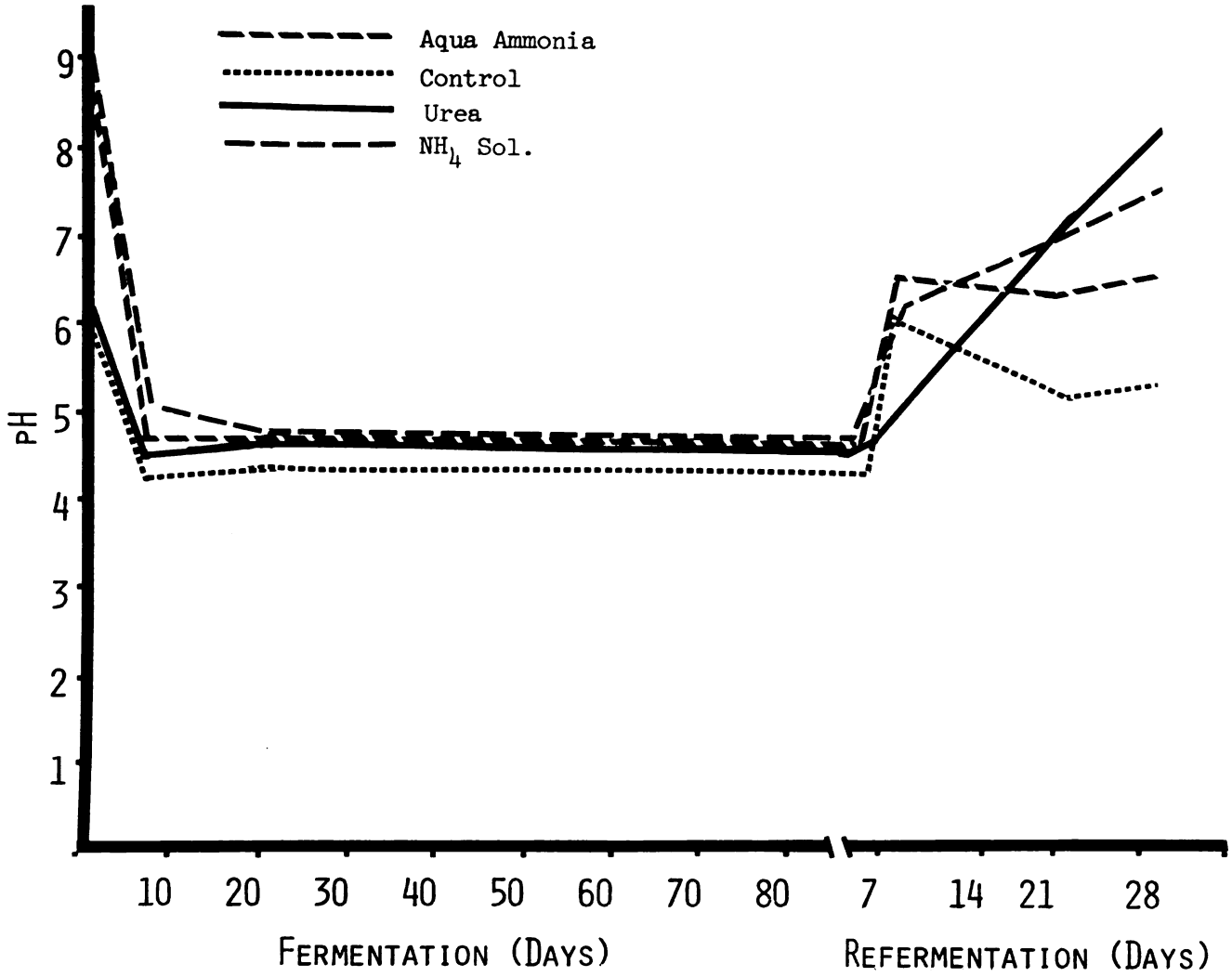

Fig. 4.--PH of Corn Silages Treated with .2% N from 3 NPN Sources

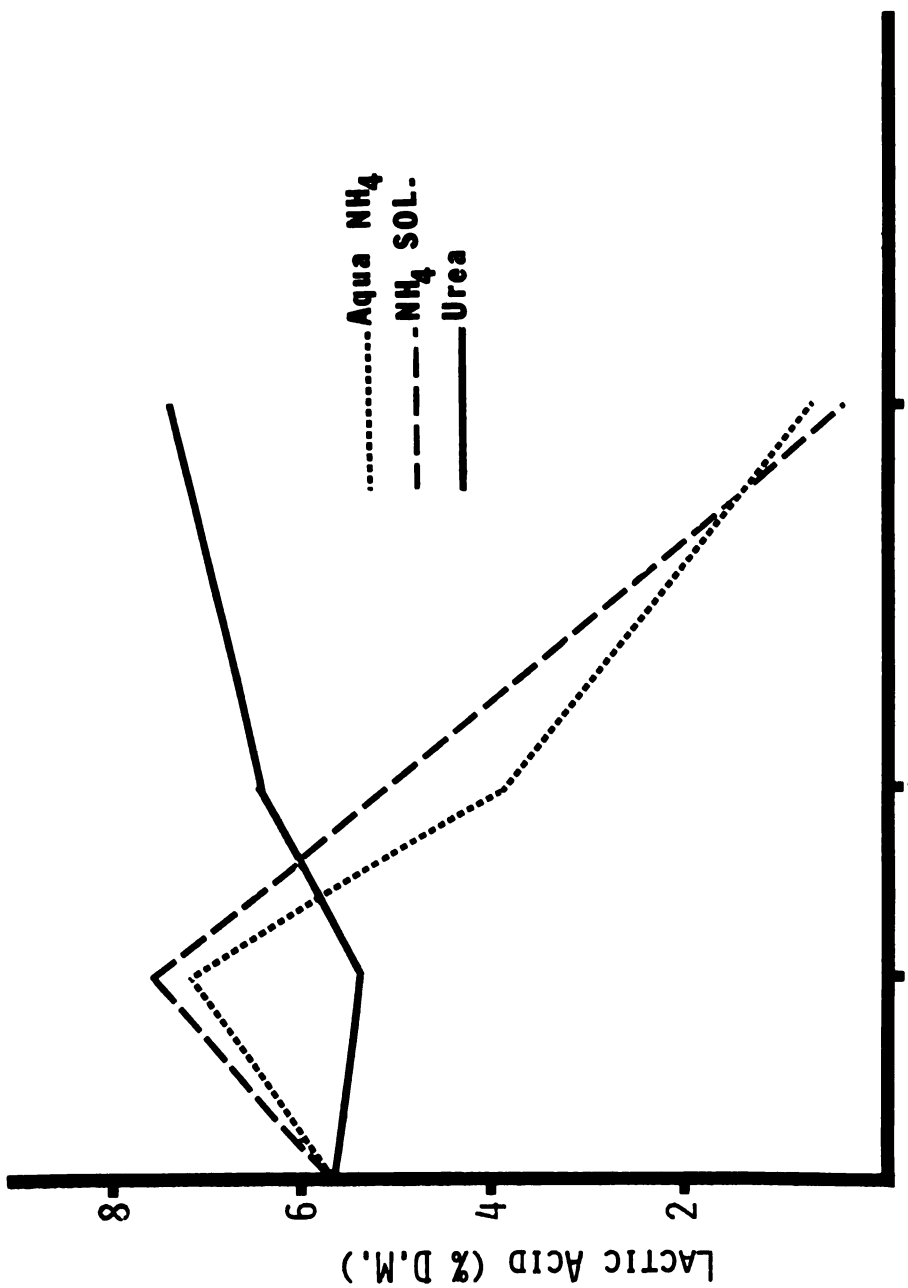

Fig. 5.--Lactic Acid Concentrations of Corn Silage Treated with Varying Levels of N from 3 Sources (averaged for 20 and 86 days of fermentation)

fermentation and late in refermentation. (Complete data for lactic acid production in Appendix, Table 2.)

Lactic acid production (Figure 6) on control and .2% urea N was almost complete 10 days after ensiling while the higher peaks for the ammonia treatments occurred on day 20. The ammonia buffered the products of fermentation, thus extending the period of lactic acid production. Lactic acid remained stable until refermentation at which time concentrations decreased to negligible levels at spoilage as reported by Sleiman (1972).

No differences in the maximum temperature during refermentation were observed between treatments or levels of nitrogen addition (Table 13). This may have been due to the small mass of the silage (12 kg) and the high constant temperature (25 C) of the storage room. Small changes in silage temperature would have been overshadowed by the room temperature. Neither were any differences seen in temperature during fermentation. Again large changes in ambient conditions (21 C and 5 C) and the small mass of silage may have masked changes. However, if complete anaerobic conditions were achieved little heating would have been expected (Federson, 1971).

Time until spoilage was observed (mold growth; Table 13) was increased at both the .2 and .4% N additions so it appears that added N as ammonia or urea (which partially degrades to ammonia) has some value in preservation of forages. At the .8% N, spoilage was fastest,

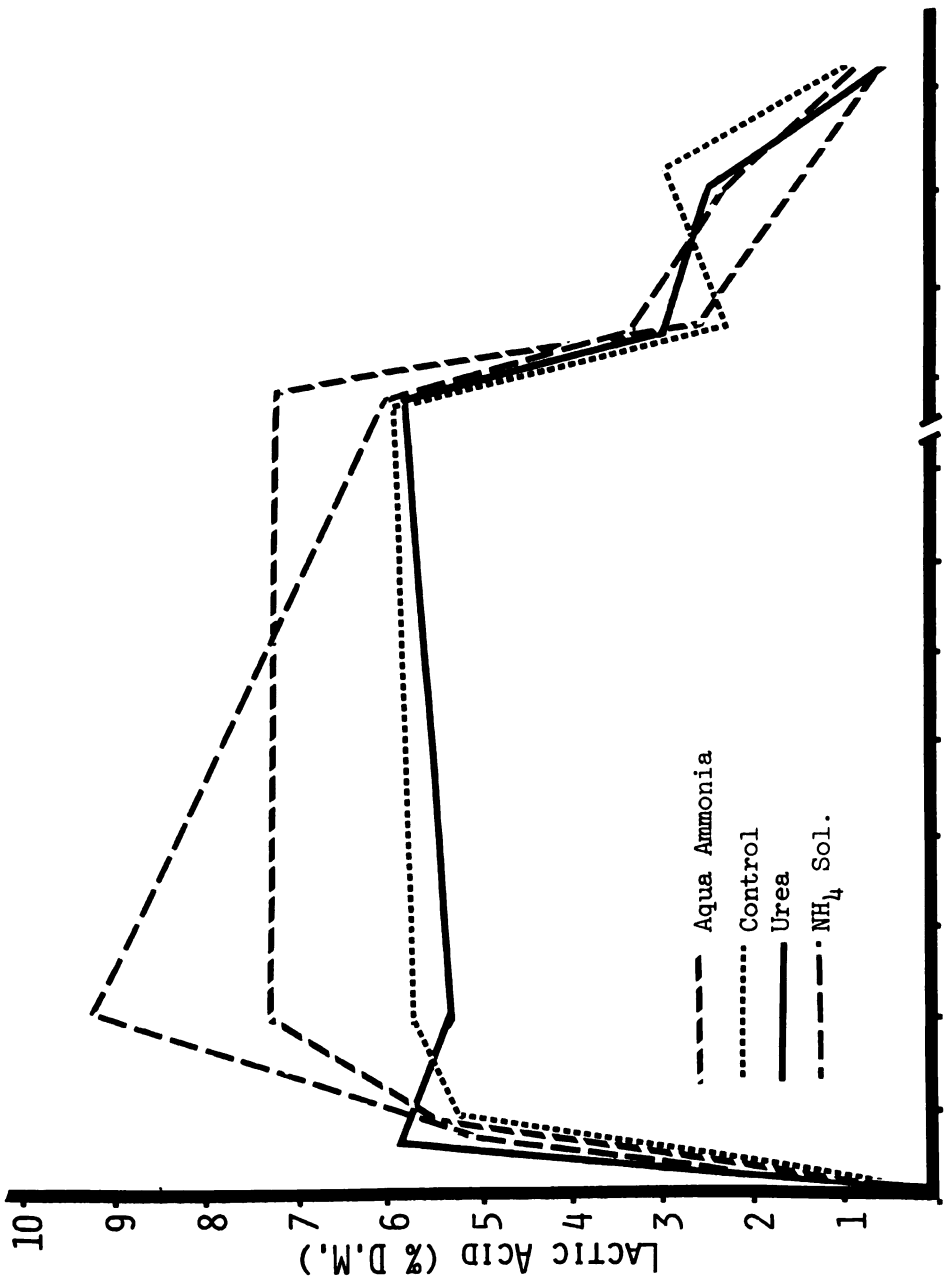

Fig. 6.--Lactic Acid in Corn Silage Treated with .2% N from 3 NPN Sources

TABLE 13.--Maximum Temperatures, Days until Spoilage and Days until Maximum Temperature During Refermentation as Affected by Different Levels and Sources of N Addition to Corn Silage

TREATMENT	MAXIMUM TEMPERATURE (C)	DAYS UNTIL SPOILAGE	DAYS UNTIL MAXIMUM TEMPERATURE
CONTROL	32.0	4.0^{A}	10.5
UREA	32.5	7.3^{AB}	15.3
NH_4-SOLUTION	33.0	12.4^{B}	16.5
AQUA-NH_4	33.4	4.6^{A}	13.0
LEVEL OF TREATMENT			
0.0	32.2	4.0	14.5
0.2	32.2	11.3	17.8
0.4	35.5	11.5	16.8
0.8	32.0	4.0	10.5

^{AB}Means not sharing same superscript are different ($P < .05$).

requiring only 4 days. Thus, the benefit of N in preservation is not due to N per se but to changes mediated through fermentation. Days until spoilage (Table 13) was increased ($P < .05$) by the NH_4-solution compared to control and aqua-NH_4. Ammonia alone appears not to be as effective a fungicide in preservation of forages as in high moisture corn (Bothast et al. 1973). However, all nitrogen treatments increased days until spoilage. Days until maximum temperature closely paralleled days until spoilage although no significant differences were found. Maximum temperatures required approximately the same amount of time after fungi were first detected regardless of the time required for mold growth (spoilage) to begin.

Ammonia was very effective in destroying fungi present in fresh-cut corn silage (Table 14) as previously reported by Bothast et al. (1973) and Tomkins and Trout (1931). The effect was apparently due to the NH_4 ion and was not exhibited by urea. However, after fermentation was initiated fungal growth was greatly decreased on all treatments which explains why no significant differences in fungal counts were seen between urea and ammonia during fermentation, which coincides with anaerobic conditions being reached (Federson, 1971).

During fermentation both ammonia treatments had an increase in fungal colonies which was larger on the higher treatment levels. (Table 14 and Figure 7) This was due to the higher pH on these treatments which inhibited lactic acid production and left sufficient

TABLE 14.--Total Fungal Colonies[A] (Total Counts/g x 10^3) in Corn Silage as Affected by NPN Treatment During Fermentation and Refermentation

	DAYS OF FERMENTATION				DAYS OF REFERMENTATION		
	0[B]	5	20	86:0	7	21	28
CONTROL	432	14	39	16	43,352	12,397	4,775
.5% UREA	2,750	26	2	39	9,422	1,024	336
1.0% UREA[C]	1,300	16	0	23	312	121	13
2.0% UREA	467	33	1	14	3,098	33	24
1.0% AQUA-NH_4	57	54	0	822	21,058	42	492
2.0% AQUA-NH_4[C]	17	48	3	1,147	9,225	13,963	14
4.0% AQUA-NH_4[C]	1	2	0	1,390	4,500	37	17
1.8% NH_4-SOL	29	18	0	865	18,300	326	251
3.6% NH_4-SOL[C]	22	10	4	377	7,750	9,488	190
7.2% NH_4-SOL[C]	1	28	4	1,175	782	31	15

[A]Each value is the average of two duplicates.

[B]Total fungal counts reduced by NH_4-solution and aqua-NH_4 30 minutes after treating ($P < .01$).

[C]Total fungal counts reduced by .4 and .8% added nitrogen ($P < .01$).

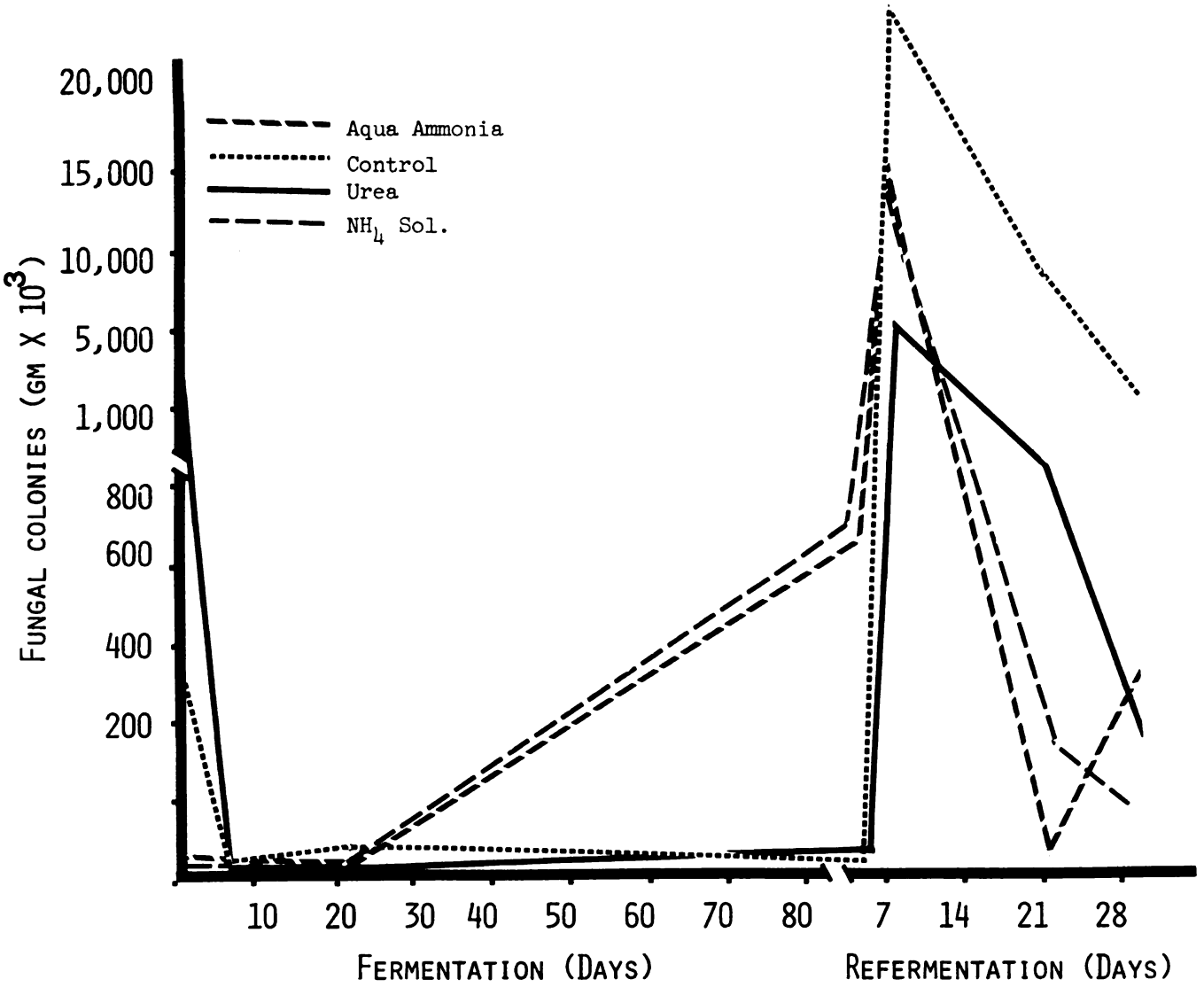

Fig. 7.--Relation of NPN Treatment of Corn Silage to Total Fungal Colonies During Fermentation and Refermentation

oxygen and water soluble carbohydrates to permit fungal growth. However, the urea and low level of ammonia N had extensive fermentation and fungal growth was comparable with controls. During ensiling normal fermentation appears to be as efficient a fungicide as ammonia or urea which is hydrolyzed to ammonia during fermentation.

Upon aeration or initiation of refermentation all silages exhibited a rapid increase in fungal numbers but thereafter fungal numbers for the ammonia-treated silages decreased at a faster rate than for the control or urea silages (Table 14 and Figure 7). There was a marked depression of fungi by all nitrogen treatments compared to the control with a significant difference ($P < .01$) being seen at the .4 and .8% N treatments; however, no treatment differences were detected. A rapid decrease in fungal numbers was noted on the ammonia treatments during refermentation due to the release of ammonia during refermentation. This effect was more pronounced on the higher nitrogen treatments (Table 14) because of more ammonia release. Fungi on the high urea treatments decreased rapidly during the later stages of refermentation indicating extensive release of ammonia. The control also exhibited a decrease in fungal numbers due to loss of lactic acid and possibly deamination of protein and a decrease in available nutrients.

Dry matter losses were 1.84, 2.55, 4.08, and 1.43% respectively for the 0.0, 0.2, 0.4, and 0.8% N treatments. The higher dry matter

losses at .2 and .4% N might have been due to a more extensive fermentation indicated by higher lactic acid.

Yeast decreased ($P < .01$) as a per cent of total population during fermentation and refermentation (Table 15) while Geotrichum increased ($P < .01$). Penicillium were isolated only at the end of fermentation and no significant numbers of Aspergillus were seen at any time. Aspergillus were not detected in spoiled material and the spoilage appeared to be due largely to bacteria because of the low fungal population (Table 14) at the end of refermentation. Geotrichum was the fungus which grew most at spoilage and was associated with visual molding. No differences were observed between treatments in type or relative proportions of fungi; hence, NPN has no apparent effect on relative types but delays overall fungal growth.

TABLE 15.--Relative Proportions of Fungi in Corn Silage Treated with Various Kinds of NPN

DATE	YEAST	GEOTRICHUM	PENICILLIUM
DAY 0	90.0%^{A}	.3%^{A}	0.0%^{B}
END OF FERMENTATION	83.3%^{A}	12.1%^{AB}	4.5%^{A}
END OF REFERMENTATION	60.3%^{B}	27.3%^{B}	0.0%^{B}

^{AB}Means in same column with different superscripts different ($P < .01$).
^{C}Insignificant number of Aspergillus found.

Summary and Conclusions

Nitrogen Treatment decreased the number of total fungi. With the exception of 30 minutes after addition no significant differences between treatments were observed during fermentation. The addition of more than .2% ammonia nitrogen severely inhibited lactic acid production and was associated with higher fungal counts before opening the silos. Ammonia increased the days until visual spoilage was detected, but no significant changes between treatments were noted for fungal types. Yeast decreased and Geotrichum increased during fermentation and refermentation. Insignificant numbers of Aspergillus and Penicillium were found.

These data indicate that in addition to supplying NPN, ammonia can be used to inhibit fungal growth and increase stability of silage when exposed to air. More than .2% nitrogen as ammonia depresses the quality of silage as indicated by decreased lactic acid production.

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V.

PART C: PRESERVATION AND ANIMAL PERFORMANCE OF HIGH MOISTURE CORN TREATED WITH NON-PROTEIN-NITROGEN AND PROPIONIC ACID

Abstract

High moisture shelled corn harvested at approximately 27% moisture was treated with: 1) propionic acid (1.2%), 2) a mixture of 80% propionic and 20% acetic acid (1.2%), 3) aqua-ammonia (at .54% NH_3) or 4) a commercial ammonia-solution (at .63% NH_3). For treatments 1, 3, and 4 approximately 150 bushels of corn was treated and stored in gravity wagons until feeding. Only 5 bushels of corn for laboratory use was treated with the propionic:acetic mixture.

Recovery of nitrogen for the ammonia treatments was 60% for the ammonia-solution and 30-40% for the aqua-ammonia while 80% of the propionic was recovered. No significant changes in protein or acid content were observed during storage. Fungi were reduced by all additives (21 vs 690 colonies/g X 10^3) 30 minutes after treatment. Fungal counts after 28 days for the aqua-ammonia and control treatments were significantly higher than the others. During late storage both ammonia treatments showed increases in fungal colonies. These occurred earlier and were of greater magnitude for the aqua-ammonia than on the ammonia-solution. After 60 days of storage corn treated with aqua-ammonia heated to 50 C while the other treatments remained at ambient temperature. After rolling the corn prior to feeding the aqua-ammonia corn

had higher fungi ($P < .05$) than other treatments. After rolling in a hammer mill, corn treated with the ammonia-solution heated to 30 C while the propionic remained at ambient temperature (4-10 C) and had not heated 60 days after rolling.

Lactating dairy cows (8 per group) readily consumed all corns in a 5-week feeding trial, even though that treated with aqua-ammonia was heavily molded. No health problems were detected in any of the cows due to eating the moldy corn. Milk production was 21.2, 18.4 and 19.3 kg for treatments 1, 3, and 4 respectively ($P < .109$) and persistencies of milk yield (trt/std) averaged 93, 88, and 81% for the respective treatments ($P < .126$).

Introduction

Changes in harvesting methods and difficulty in drying wet corn has created increased interest in high moisture (HMC). The storage of HMC has caused problems of molding. Trenk and Hartman (1968) concluded that at moistures above 17.5% and temperatures greater than 13 C corn was susceptible to aflatoxin formation by *Aspergillus flavus*. Lynch *et al*. (1969) found a 6% decline in milk production when hay molded with *Aspergillus* and *Penicillium* was fed to cows and Mirocha *et al*. (1968) reported that feeding moldy hay to

150 dairy cows increased the number of services per conception from 1.2 to 4.0.

Preservation of HMC with organic acids is now competitive with drying. Porter (1946) found organic acids had a greater microbiological inhibitory effect at the same pH than mineral acids which was attributed to the whole molecule. Richardson and Halick (1957) found propionic acid was fungicidal in corn meal at .1%, while .3% calcium propionate was required for equal protection and sodium propionate was not effective at .6%. Propionic acid prevented mold growth in HMC (Jones, 1970; Arends et al. 1970).

An even less expensive method of preserving HMC was reported by Bothast et al. (1973). They treated 12% moisture and shelled corn reconstituted to 26% with ammonia at 2 or .5% of dry weight. Both concentrations of ammonia eliminated external and infecting molds and yeasts.

The objective of this study was to compare ammonia, and propionic acid as a fungicide for HMC. The nutritive values of treated grains for lactating dairy cows was also ascertained.

Materials and Methods

TRIAL I: High moisture shelled corn (HMC) containing approximately 27% moisture was harvested in January, 1973 and treated with propionic acid (1.2%) or an 80% propionic, 20% acetic mixture (1.2%) called chemstor.[A] Acids were sprayed onto corn as it passed through an auger designed for that purpose.[A] Two additional lots of corn were treated with aqua-ammonia (aqua-NH_4; 22%N) applied at .54% NH_3, or an ammonia-molasses-minerals mixture[B] (13% N; NH_4-solution) applied at .63% NH_3. The ammonia materials were added to the corn as it entered a forage blower after metering through a calibrated pump. Approximately 150 bushels of corn were treated with propionic, aqua-NH_4, or the NH_4-solution and augered into gravity wagons until fed. Only about 5 bushels was treated with chemstor which was sufficient for laboratory tests.

From all treatments 35 kg portions of HMC were placed in open polyethylene containers and stored at 25 C. Temperature VFA, lactic acid, protein, DM, pH, and fungal counts and identification were measured on samples collected on days 0, 2, 5, 9, 16, 23, 30, and 37 of storage. Samples were stored at -20 C until analyzed. After 90

[A]Furnished by Celanese Chemical Co., Corpus Christi, Texas.

[B]Furnished by Ruminant Nitrogen Co., Adrian, Michigan (Trade name--Prosil).

days of storage in the wagons, additional 35 kg portions were placed in a 25 C room as previously described and sampled at days 0, 3, 7, 10, 17, and 31.

Rolling HMC before feeding is generally recommended. The HMC was removed from the storage wagons and passed through a roller mill in preparation for feeding to dairy cows. Rolled corn was placed in 200 ℓ barrels and stored in a barn at ambient temperatures. Samples were collected on days 0, 4, 6, 9, 12, and 13 and analyzed as previously outlined.

Corn samples remained frozen at -20 C until analyzed. For DM determination corn was ground and 20 g were placed in a forced air oven at 90-100 C for 24 hours. A homogenate for pH, lactic acid, VFA, and fungal analyses was prepared by blending a 40 g aliquot of HMC with 160 ml of distilled sterile water for 3 minutes in an omni-mixer[A] with the cup immersed in ice. Approximately 50 ml of the homogenate was placed in a separate sterile container for plating of fungal populations. The pH was measured with a Sargent pH meter.[B] Extracts were prepared by straining the homogenate through two layers of cheesecloth. Extracts were deproteinized with 50% SSA and analyzed for lactic acid, VFA, and fungi as described in Part A for corn silage.

[A]Ivan Sorvall Inc., Newton, Connecticut.

[B]E. H. Sargent and Co., Chicago, Illinois.

TRIAL II: The rolled HMC from the storage wagons in Trial I was fed for 5 weeks to 24 lactating dairy cows averaging between 14 and 36 kg of milk per day. Cows were blocked according to production during pre-treatment and randomly assigned to the three HMC feeds. The HMC was fed at 1 kg per 2.5 kg of milk produced during pre-treatment, corn silages at 11.4 kg per day and alfalfa haylage ad lib. During the experimental period corn silage, haylage, and HMC were sampled 3 times weekly. Samples were composited on a weekly basis and frozen for analyses. Orts were measured daily and daily feed, DM, and protein intake were determined. Milk production and butterfat percent were calculated on a weekly basis.

Results and Discussion

Trial I: The acids caused no visual change in the color of corn kernels while both ammonia treatments darkened the seeds immediately after application. It was planned to apply the ammonia at equal rates but due to difficulties in the calibration of the pump the NH_4-solution was applied at .63% NH_3 (.85% of DM) and the aqua-NH_4 at .54% NH_3 (.73% of DM). Bothast et al. (1973) reported that addition of .5% NH_4 (of dry weight) to 27% moisture corn destroyed

both external and infecting fungi. Hence, the level of ammonia in my study should have prevented fungal growth.

Organic acid levels (1.2%) were within the range which inhibited fungal growth in 27% moisture corn (Miller, 1971). The amount of propionic acid needed for preservation was directly proportional to the moisture content of the HMC.

Temperatures of grain in the wagons were checked periodically. After 60 days of storage, temperatures of the aqua-NH_4 corn increased above the ambient (7-14 C) and reached 40-50 C within 10 days; while temperatures of corn treated with propionic and NH_4-solution remained at about ambient. Heating forced the feeding trial to be initiated earlier than had been planned. These data show that the aqua-NH_4 was ineffective in preventing heating in stored HMC for 60 days, although it was effective for approximately 30 days during which time control corn became heavily molded. Bothast et al. (1973) found ammonia treatment preserved corn for 14-21 days. Propionic acid and the NH_4-solution prevented heating for the entire period the HMC was stored prior to feeding.

To test the preservative effects of NPN and organic acids under better controlled storage than gravity wagons, 35 kg portions were placed in polyethylene containers and stored at 25 C. In these samples no lactic acid was detected at any date. A lack of fermentation was also implied by the pH values (Table 16), which did not

TABLE 16.--The pH During Storage of HMC Treated with Organic Acids or NPN

	Days of Storage			
	2	9	23	30
PROPIONIC[A]	4.50	4.50	4.50	4.50
CHEMSTOR[A]	4.55	4.55	4.55	4.60
AQUA-NH_4[B]	7.85	7.35	6.70	6.70
NH_4-SOL[C]	9.00	8.45	8.35	8.35
CONTROL[D]	6.20	6.45	6.20	6.35

[ABCD]Treatments not sharing same superscripts significantly different ($P < .01$).

change during storage. The acid additions significantly decreased pH ($P < .01$) compared to untreated corn; while the ammonia increased pH, with the NH_4-solution higher ($P < .01$) than the aqua-NH_4 due to a higher level of application.

The nitrogen content of the HMC was increased more by the NH_4-solution than the aqua-NH_4 (Table 17) due to the higher rate of application and greater apparent loss of aqua during treatment (66 vs 40%). The high apparent loss of nitrogen may have been due to miscalculated flow rates and corn weight. Hence, it is impossible to accurately determine the true loss of ammonia during treatment. There was no

TABLE 17.--Crude Protein (% of DM) of HMC During Storage Treated with NPN and Organic Acids

	Days of Storage			
	2[D]	9[D]	23[DE]	30[E]
PROPIONIC[A]	10.25	9.89	10.25	10.34
CHEMSTOR[A]	9.79	9.56	10.44	10.07
AQUA-NH_4[1][B]	11.27(34)	11.11(26)	11.44(39)	11.39(57)
NH_4-SOL[1][C]	12.06(48)	12.50(58)	12.63(61)	13.16(73)
CONTROL[A]	9.90	10.11	9.90	10.25

[ABC]Treatments not sharing the same superscripts are significantly different ($P < .01$).

[DE]Means not sharing the same superscripts significantly different ($P < .01$).

[1]Percent recovery of nitrogen or the NH_4 treatments given in parenthesis.

apparent loss of nitrogen (ammonia) during storage. In fact, after 30 days of storage protein was significantly higher on all treatments than at 2 days. After adding to HMC, ammonia is tightly bound, so the poor initial recoveries are not due to losses after treatment. Some losses did occur during treatment because of the presence of an ammonia odor in the area of treatment; however, the accurate determination of the extent of these losses cannot be determined from these data.

Estimated recoveries of propionic acid (Table 18) averaged 8% on the propionic treatment and 82% for chemstor. Five of eight determinations indicated almost total recovery and differences in individual sampling may explain the low estimates (41 and 63%) obtained in two samples.

TABLE 18.--Propionic Acid (% wet wt) of HMC Treated with NPN and Organic Acids After Storage

	Days of Storage			
	3	9	23	30
PROPIONIC[1]	1.25(100)	.76(63)	1.17(98)	1.11(93)
CHEMSTOR[1]	.39(41)	1.12(117)	.91(95)	.72(75)
AQUA-NH_4	.02	.06	.02	.08
NH_4-SOL	.06	.11	.08	.01
CONTROL	.02	.08	.02	.06

[1]Percent recovery of propionic given in parenthesis.

Total fungi were significantly depressed by all treatments (Figure 8; complete data in Appendix, Table 3). Thus organic acids and ammonia were equally effective in destroying initial fungi. By 7 days fungal counts in both ammonia treatments began to increase, but at 28 days, corn treated with the NH_4-solution reverted back to counts similar to those for the acid treatments; while those for aqua-NH_4 continued to increase. Corresponding to the higher fungal counts in the warm room; the aqua-NH_4 corn was the first to heat in the gravity wagons, exposed to colder ambient temperatures. Gilman and Barron (1930) found that heating of grains was due primarily to fungal growth.

The decreased mold inhibition on aqua-NH_4 may have been due to loss of the NH_4 ion from the internal atmosphere of the corn because of a possible linkage with carbohydrates. Tomkins and Trout (1931) reported that 500-1000 PPM of ammonia in the atmosphere was required to prevent fungal growth. Loss of the ammonia odor from the grain occurred about the time heating and fungal growth began. Bothast et al. (1973) reported that ammonia killed initial fungi in HMC as in this data but after ammonia disappeared from the atmosphere surrounding the HMC, spores were still present to initiate new fungal growth which was largely uninhibited (Christensen and Gordon, 1948). Thus, ammonia appears to be a very effective short-term grain preservative. Its value is rapidly lost during storage under minimal protection.

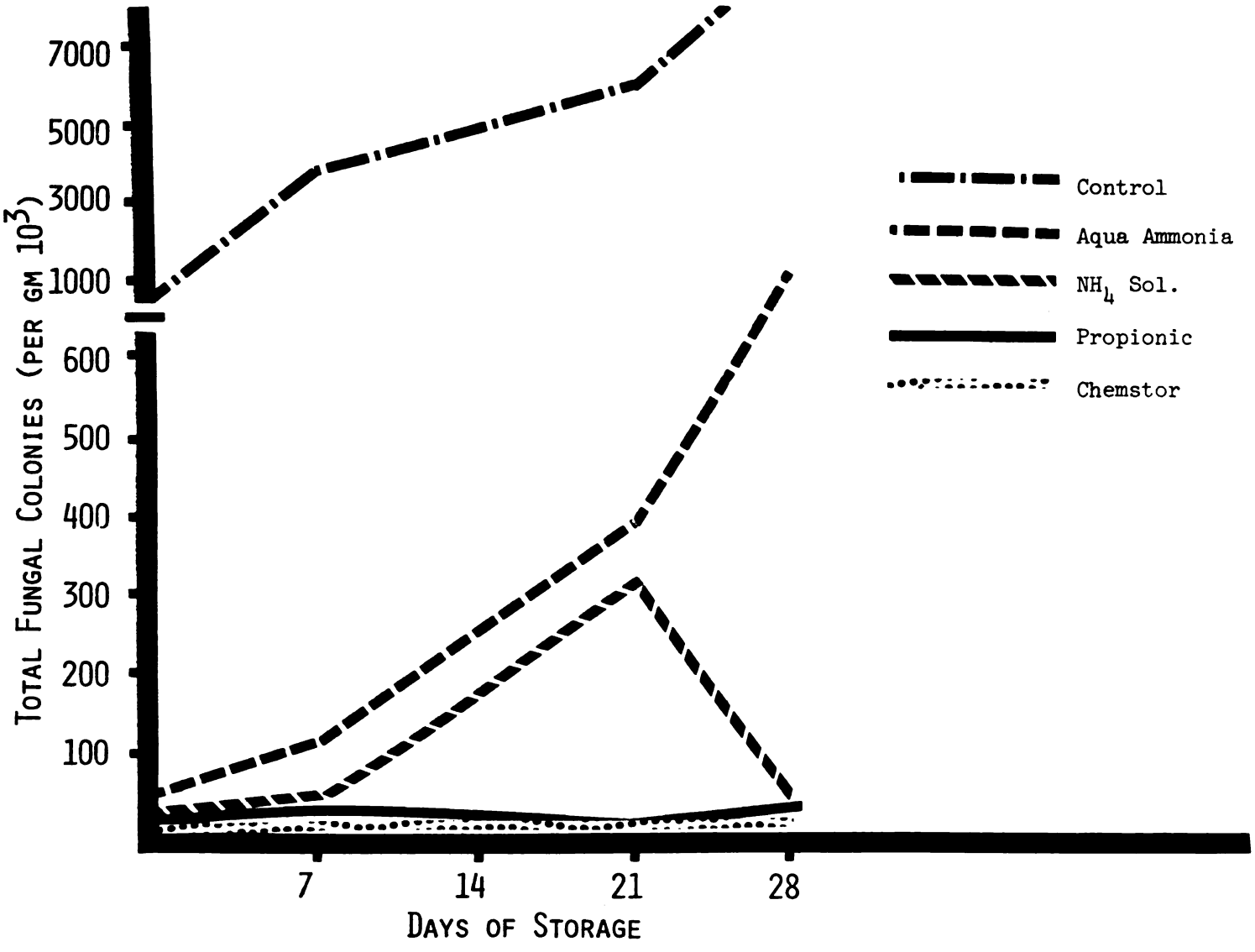

Fig. 8.--Fungal Counts of HMC Treated with Ammonia and Organic Acids During Storage

Another possible explanation for the poorer fungicidal action of aqua-NH_4 was the low ammonia level (.31% of the corn DM) in this treatment, whereas the NH_4-solution contained (.51% of DM) similar to that shown to have fungistatic properties by Bothast et al. (1973).

Relative proportions of fungi did not change and spoilage was largely due to yeast and Geotrichum; however, some Aspergillus and Penicillium were observed. The effectiveness of propionic acid in preventing fungal growth agrees with Porter (1946), Richardson and Halick (1957), Weise (1971), and Jones (1971). There were numerous fungi present at harvest since the corn had been standing in the field ready to harvest for 90 days. The propionic was fungicidal to this population in support of Singh-Verma (1971) who found the acid most effective after deterioration had begun because cells were growing in their log phase.

The next two sections of Trial I are very similar to the previous section and the data will only be discussed as it contradicts data from the first section. The second section compared aqua-NH_4, NH_4-solution, and propionic acid treatments during refermentation. This was corn which was treated and stored as described in section 1 and removed from the gravity wagons (after 60 days) at initiation of this experiment.

The pH of propionic-treated HMC remained constant after removal from the gravity wagons throughout the entire period (Table 19)

TABLE 19.--PH and Protein[1] (% of DM) of HMC Treated with NPN and Organic Acid During Refermentation

	Days of Refermentation				
	0^{D}	3^{DE}	7^{DE}	10^{E}	17^{E}
CRUDE PROTEIN					
AQUA-NH_4[1]	11.44(39)	11.43(39)	11.42(39)	11.92(52)	11.99(54)
NH_4-SOL[1]^{B}	12.44(56)	12.61(60)	12.12(49)	12.53(59)	12.53(59)
PROPIONIC^{C}	10.16	10.17	10.14	10.31	10.21
PH					
AQUA-NH_4^{A}	7.90	7.67	7.40	7.23	6.80
NH_4-SOL^{B}	8.40	8.28	7.82	7.50	7.85
PROPIONIC^{C}	4.65	4.65	4.65	4.58	4.67

^{ABC}Treatments not sharing same superscripts different ($P < .01$).

^{DE}Dates not sharing same superscripts different ($P < .05$).

[1]Nitrogen recoveries (%) in parentheses.

while that of ammonia treatments significantly decreased ($P < .05$) during refermentation indicating production of acids or loss of the additive. The ammonia had higher pH values than the propionic ($P < .01$) and the aqua - NH_4 was lower ($P < .01$) than the NH_4-solution. Crude protein was also higher ($P < .01$) for the ammonia treatments with the NH_4-solution higher than aqua. Nitrogen recoveries averaged 56.6% for the NH_4-solution and 44.6 for aqua. No apparent losses occurred over storage so the decreased pH was probably due to acid production. Neither was there loss of propionic acid after 3 days of storage (Table 20); however, there appears to be a loss between days 0 and 3 of storage which may have been due to sampling error.

TABLE 20.--Propionic Acid (% wet wt) of HMC Treated with NPN and Organic Acids During Refermentation

	Days of Refermentation				
	0	3	7	10	17
AQUA-NH_4	.07	.05	.05	.06	.06
NH_4-SOL	.16	.08	.09	.08	.03
PROPIONIC[1]	1.26(1.05)	.90(75)	.80(67)	.88(73)	.89(74)

[1]Propionic acid recoveries (%) in parentheses.

Fungal data is given in the Appendix (Table 4). Fungal growth (Figure 9) began to increase in 3 days on the aqua-NH_4 and at 10 days on the NH_4-solution while remaining at near zero on the propionic treatment. At 31 days the ammonia treatments had significantly more ($P < .10$) fungi than the propionic. Geotrichum and yeast were again responsible for spoilage, while some Aspergillus and Penicillium were detected. No differences in temperatures were observed.

In section 3 the corn was rolled and 120 kg portions were placed in steel drums for storage prior to feeding. Because of a mechanical problem all the propionic-treated HMC had to be rolled at the initiation of the trial but no heating or fungal growth was evident during the 40 days of feeding while the ammonia-treated HMC was rolled twice weekly. The pH values for the propionic treatment (Table 21) remained stable throughout storage while the ammonia treatments decreased, reflecting loss of ammonia or formation of an ammonia-carbohydrate complex. The protein content remained stable throughout storage so formation of a complex appears unlikely. The NH_4-solution treated corn had higher pH and protein than the aqua ($P < .01$). Propionic acid recoveries after rolling were much higher than in either of the two previous studies averaging 167% (Table 22). The only explanation is that rolling stimulated propionate fermentation which is not likely.

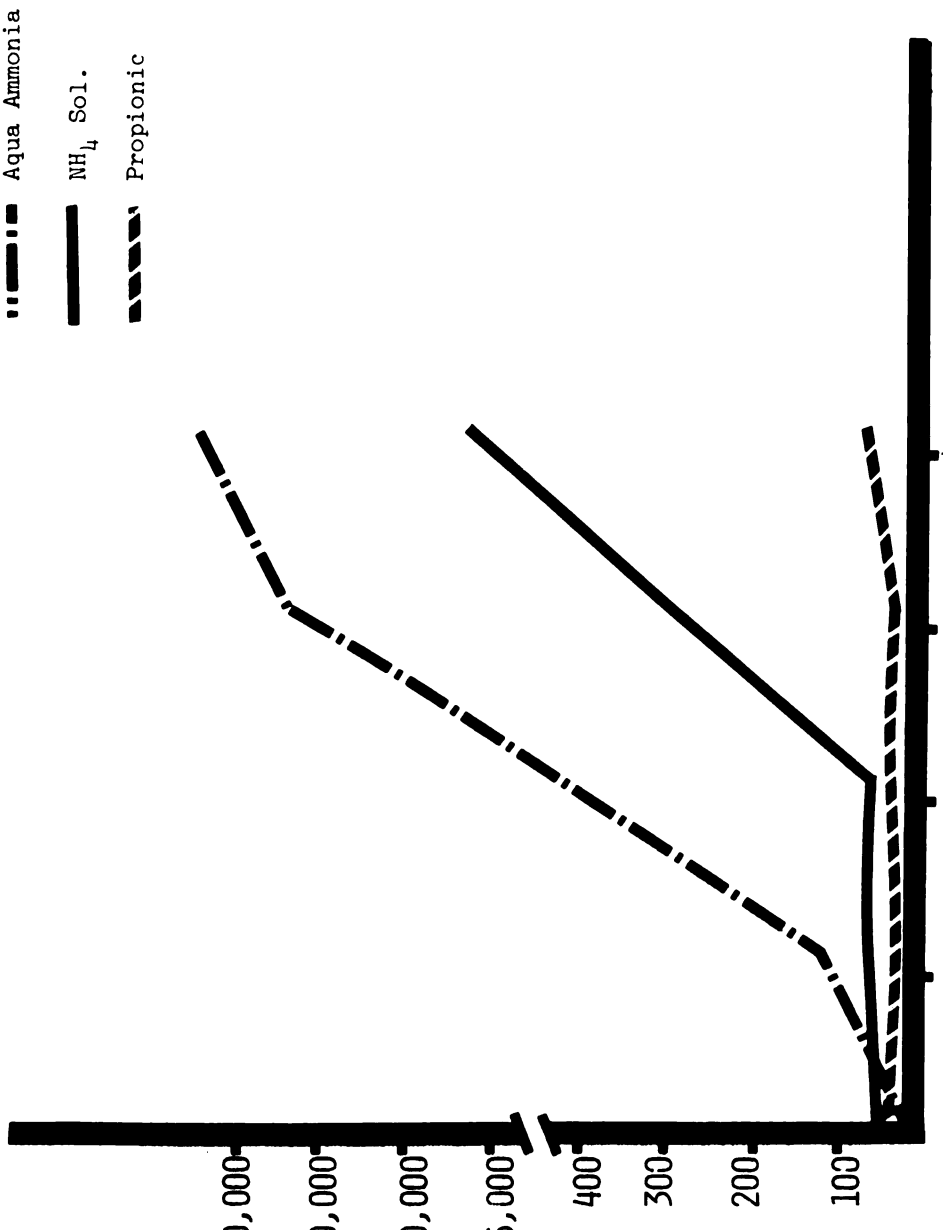

Fig. 9.--Relationship of Days of Storage During Refermentation to Fungal Growth in Propionic and Ammonia Treated HMC

TABLE 21.--PH and Crude Protein[1] after Rolling of HMC Treated with NPN and Organic Acids

	Days after Rolling					
	0	4	6	9	12	13
			-------- PH ---------			
PROPIONIC[A]	4.60	4.75	4.60	4.70	4.75	4.65
AQUA-NH_4[B]	8.00	7.20	7.45	7.50	7.45	7.60
NH_4-SOL[C]	8.55	8.15	8.35	8.25	8.25	8.10
			--------- CRUDE PROTEIN --------			
PROPIONIC[A]	10.18	10.16	10.32	10.45	10.46	10.15
AQUA-NH_4[B]	12.12(57)	11.84(50)	12.31(62)	11.98(53)	11.80(49)	11.87(51)
NH_4-SOL[C]	13.03(70)	12.76(64)	12.73(63)	13.05(70)	13.12(72)	13.05(70)

[1]Percent nitrogen recoveries in parenthesis.

TABLE 22.--Propionic Acid (% Wet Wt) of HMC After Rolling Treated with NPN and Organic Acids

	Days After Rolling					
	0	4	6	9	12	13
PROPIONIC[1]	1.94(162)	2.03(169)	1.85(154)	2.14(178)	2.21(184)	1.89(158)
AQUA-NH_4	-	.07	.12	.22	.08	.07
NH_4-SOL	.15	.10	.10	.12	.27	.07

[1]Figures in parentheses are % propionate recoveries.

The aqua-NH_4 corn which had heated in the wagons was significantly higher in fungi ($P < .01$) after rolling and throughout storage (Figure 10). Fungi in the NH_4-solution increased when compared to propionic but no significant differences were seen. Fungal data is in Appendix (Table 5).

The temperature (Figure 11) of the rolled HMC treated with propionic acid remained close to ambient while the temperature of the aqua-NH_4 which had peaked in the wagons decreased. After three days the temperature of the NH_4-solution HMC increased until day 7 then began to decrease. This heating closely parallels the increase observed in fungal counts during the storage period and confirming the role of fungi in grain heating. No differences in type of fungi were noted between treatments.

Trial II: As indicated by storage data, heating of the aqua-NH_4 treated HMC necessitated beginning feeding earlier than anticipated. Because of this, cows had not been on a standardization ration so they were allotted to treatment according to milk yields for the 28 days before this trial started. No fat tests were available during the period so it was not possible to co-vary fat corrected milk (FCM) during the trial and pre-treatment values as is usually done.

Milk production (Table 23) adjusted for pre-treatment differences was depressed on aqua-NH_4 ($P < .109$). There was also a decline in fat percent of cows on aqua-NH_4. Adjustment of this data to FCM

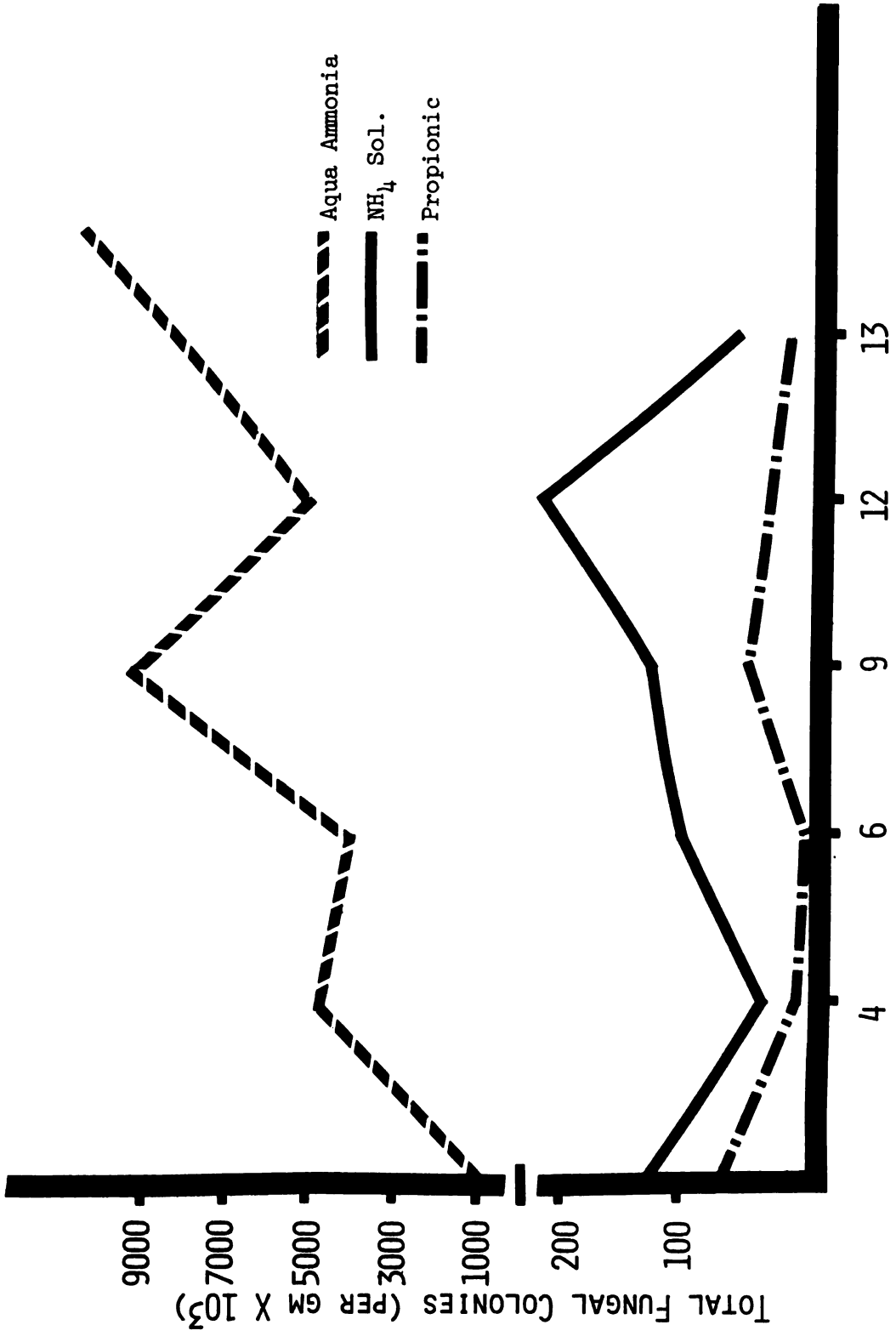

Fig. 10.--Fungal Colonies after Rolling in Ammonia and Propionic Treated HMC

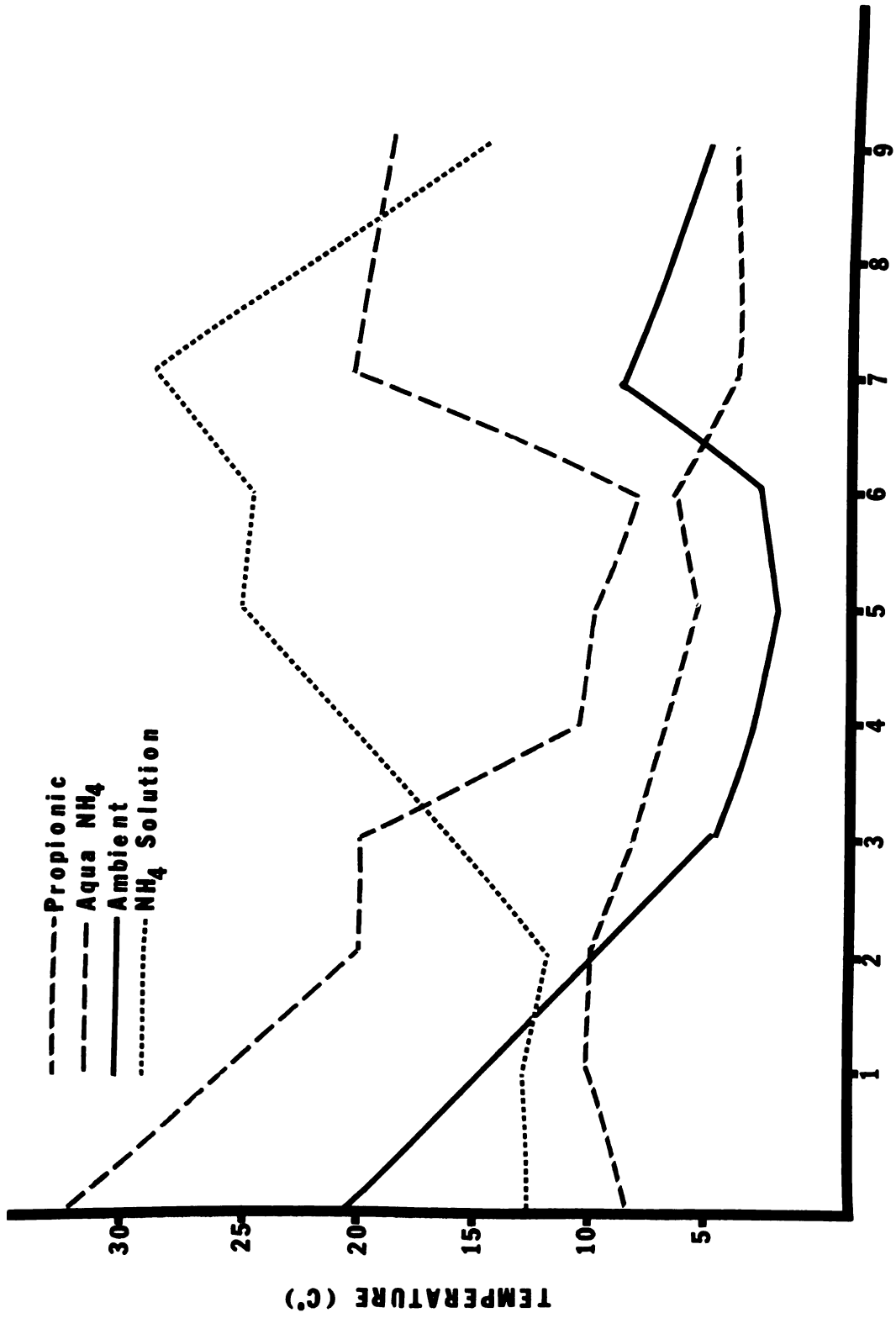

Fig. 11.--Temperature Development after Rolling in HMC Treated with Ammonia or Propionic Acid

TABLE 23.--Milk Production Data of Cows Fed HMC Treated with Either Aqua Ammonia, Propionic Acid, or Ammonia Solution

	TREATMENT			
	PROPIONIC ACID	AMMONIA SOLUTION	AQUA AMMONIA	LEVEL OF SIGNIFICANCE
MILK (COV. PRETRT.;kg)	21.2	19.3	18.4	.109
FAT (%)	3.56	3.53	3.37	.654
PERSISTENCY (PRETRT;%)	93.3	88.3	81.1	.126

would have probably shown a significant depression in FCM yield on aqua-NH_4. There was also a decline in milk yield persistencies on the aqua-NH_4 which approached significance ($P < .126$).

No intake (Table 24) or health problems were noted in animals fed corn treated with ammonia, despite the extensive molding which had occurred. Intake of corn DM averaged 7.0, 6.6, and 6.6 kg/day for acid, NH_4-solution and aqua-NH_4 treatments, respectively. HMC treated with ammonia had a faint ammonia odor and smelled moldy while the propionic had an acid smell, which apparently was not objectionable to the cows. Neither were total DM intakes different between groups, but protein intake was higher on the ammonia than the other treatment, because of the added nitrogen. Body weight gains were higher also on the ammonia treatments. Christensen et al. (1973) also reported no

TABLE 24.--Body Weight Gain and Feed Consumption of Cows Fed Propionic Acid, Aqua Ammonia or Ammonia Solution Treated HMC*

	PROPIONIC ACID	AMMONIA SOLUTION	AQUA AMMONIA
BODY WT GAIN (kg)	14.2	26.0	24.5
HMC INTAKE (kg DM)	7.0	6.6	6.6
TOTAL DM INTAKE (kg)	16.3	16.5	16.3
TOTAL PROTEIN INTAKE (kg)	1.93	2.16	2.10

*No means were significantly different ($P < .10$).

harmful effects on animals after feeding grain which was heavily infested with a toxin-producing strain of Aspergillus flavus.

Summary and Conclusions

Ammonia treatment of HMC will reduce fungi; however, regrowth of fungi may occur. The lower apparent recoveries of ammonia from aqua-NH_4 (30-40%) than from the NH_4-solution (60%) were probably responsible for the aqua treatment spoiling earlier. Propionic acid was a very effective preservative and approximately 85% of that added was recovered after storage at 25 C for 30 days. No apparent toxicity

was observed after feeding the moldy HMC to lactating dairy cows. The decrease in milk production on the aqua-NH_4 treatment approached significance. Ammonia does not appear to be as effective a preservative as propionic acid for HMC stored under minimal protection. However, corn retaining at least .5% NH_3 did not heat or spoil in gravity wagons for about 100 days after harvest, while that with a lesser ammonia concentration and control corn spoiled much more rapidly.

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VI

SUMMARY AND CONCLUSIONS

Experiments were conducted with corn silage and high moisture shelled corn to evaluate the preservative value of organic acids and NPN additives on the fungal growth during fermentation and refermentation of the respective feeds.

Organic acids were effective in decreasing refermentation in high moisture feeds. Propionic appears much more effective than formic acid in feed preservation. The addition of formic acid to propionic decreased the effectiveness of the propionic. Propionic acid significantly ($P < .01$) increased the number of days until spoilage. The addition of organic acids did not change the type of fungal flora present but delayed its growth. All levels of formic acid severely inhibited lactic acid production while the inhibition on the propionic treatment was small at the .5% treatment level but became severe at 2%. At the present cost of acid it appears to be a suitable additive for decreasing spoilage and refermentation.

Nitrogen treatment decreased the number of total fungi. With the exception of 30 minutes after addition no significant differences between treatments were observed during fermentation. The addition

of more than .2% ammonia nitrogen severely inhibited lactic acid production and was associated with higher fungal counts before opening the silos. Ammonia increased the days until visual spoilage was detected, but no significant changes between treatments were noted for fungi types. Yeast and Geotrichum increased during fermentation and refermentation. Insignificant numbers of Aspergillus and Penicillium were found.

These data indicate that in addition to supplying NPN, ammonia is effective in inhibiting fungal growth and increases the stability of silage when aerated. More than .2% nitrogen as ammonia depresses the quality of silage as indicated by decreased lactic acid production.

Recovery of nitrogen on the ammonia treatments of the high moisture corn averaged 60% for the ammonia-solution and 36% for the aqua-ammonia while 80% of the propionic acid was recovered. Fungi were reduced by all additives 30 minutes after treatment. After 28 days fungal colony counts for the aqua-ammonia and control treatments were higher than others. During late storage both ammonia treatments increased in fungal populations but they occurred earlier and were of greater magnitude on aqua-ammonia. After 60 days of storage aqua-ammonia treated corn heated to 50 C. Upon rolling, corn treated with the ammonia-solution heated while propionic corn remained at ambient temperatures. Lactating dairy cows consumed all corn readily, even

though the ammonia-treated corn was molded, and no health problems were noted. Milk production and persistency were less ($P < .109$) on the aqua-ammonia than other treatments.

Although ammonia increased the stability of corn silage during refermentation, it appears unsatisfactory as a long-time preservative for high moisture corn stored under minimal protection. Propionic acid treatment appears very satisfactory for preserving HMC and no heating or fungal growth occurred during storage and feeding. No acute mycotoxin poisoning of ruminant animals after eating heavily molded corn were noted.

APPENDIX

APPENDIX

TABLE 1.--PH's[A] in NPN Treated Corn Silage During Fermentation and Refermentation.

	Days of Fermentation				Days of Refermentation		
	0	5	20	86:0	7	21	29
Control	5.73	4.13	4.20	4.13	5.90	5.03	5.13
.5% Urea	6.25	4.35	4.48	4.35	4.70	6.85	7.93
1.0% Urea	6.95	4.73	4.63	4.50	4.65	6.83	6.78
2.0% Urea	6.35	4.98	4.90	4.53	4.58	6.78	7.33
1% Aqua-NH_4	8.68	4.85	4.48	4.33	5.88	6.78	7.38
2% Aqua-NH_4	9.38	6.85	5.30	4.88	5.40	6.43	7.53
4% Aqua-NH_4	9.88	9.30	7.75	6.60	8.13	8.18	7.08
1.8% NH_4-Sol	8.40	4.55	4.38	4.35	6.40	6.18	6.45
3.6% NH_4-Sol	9.08	6.95	5.05	5.13	5.03	5.88	6.15
7.2% NH_4-Sol	9.65	8.70	6.87	6.63	7.23	8.60	7.40

[A]Each value is the average of two duplicates.

APPENDIX

Table 2.--Lactic Acid Production[A] (% of DM) in NPN Treated Corn Silage During Fermentation and Refermentation.

	Days of Fermentation				Days of Refermentation		
	0	5	20	86:0	7	21	28
Control	0.22	4.06	5.61	5.81	2.25	2.78	0.84
.5% Urea	0.16	5.74	5.16	5.69	2.97	2.35	0.50
1.0% Urea	0.06	4.71	6.44	5.45	5.12	3.05	0.95
2.0% Urea	0.15	5.05	7.12	7.74	8.63	4.09	1.01
1% Aqua-NH_4	0.16	4.62	7.20	7.10	2.41	2.37	0.50
2% Aqua-NH_4	0.19	0.97	3.61	4.28	4.47	2.01	0.85
4% Aqua-NH_4	0.06	0.00	0.90	0.65	0.63	0.60	0.45
1.8% NH_4-Sol	0.43	4.64	9.21	5.94	3.14	2.20	0.75
3.6% NH_4-Sol	0.12	0.46	5.77	4.61	3.55	1.17	0.86
7.2% NH_4-Sol	0.03	0.00	0.29	0.45	0.39	0.30	0.43

[A]Each value is the average of two duplicates.

APPENDIX

TABLE 3.--Total Fungal Colonies (per g x 10^3) of HMC Treated with Ammonia and Organic Acids

	2	9	23	30
PROPIONIC	23	11	7	25
CHEMSTOR	5	4	6	13
NH_4-SOLUTION	11	46	310	41
CONTROL	690	3,800	6,000	11,800
AQUA-NH_4	450	110	390	1,005

APPENDIX

TABLE 4.--Total Number of Platable Fungal Colonies (X 10^3 per g) in HMC Treated with Various Preservatives During Refermentation

	Days of Storage				
	0	3	7	10	17
AQUA-NH_4	3	94	415	22,250	32,250
NH_4-SOLUTION	30	43	32	273	6,400
PROPIONIC	21	11	16	8	46

APPENDIX

TABLE 5.--Total Fungal Colonies (per g x 10^3) in HMC Treated with Ammonia and Propionic Acid After Rolling

	Days After Rolling					
	0	4	6	9	12	13
AQUA-NH_4	1,060	4,750	4,050	9,150	5,000	8,150
NH_4-SOLUTION	123	35	106	124	210	52
PROPIONIC ACID	69	8	3	46	29	8

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