CHEMICAL PRESERVATION OF ALFALFA HAY
FOR DAIRY COWS

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Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
December, 1978

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ACKNOWLEDGMENTS

The author wishes to express his sincere thanks and appreciation to Dr. L. J. Bush for his assistance and patience during the course of this study, and his invaluable guidance in the preparation of this manuscript. Appreciation is also extended to Dr. J. V. Whiteman, Dr. M. E. Wells and Dr. G. V. Odell for their assistance in the course of this study.

Recognition is extended to author's mother, Mrs. Mojeza Jafri, for her support and encouragement throughout the course of study. Finally, appreciation is expressed to author's wife, Naheed, daughter, Fazia, and son, Nadeem, for making this effort worthwhile.

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NOMENCLATURE

ADF - Acid Detergent Fiber

ADF-N - Acid Detergent Fiber Nitrogen

NDF - Neutral Detergent Fiber

IVDMD - _In Vitro_ Dry Matter Disappearance

DMD - Dry Matter Digestibility

VFA - Volatile Fatty Acids

Wk - Week(s)

Hr - Hour(s)

C - Centigrade

TP - Total Protein

NRC - National Research Council

MS - Mean Square

TP - Total Protein

NFS - Non-Fat Solids

CHAPTER I

INTRODUCTION

Alfalfa hay is an important forage to many dairymen in Oklahoma and many other parts of the world. However, at times it is extremely difficult to harvest alfalfa hay with the high quality desired for high producing dairy cows. Morrison (1958, p. 217) stated, "Good alfalfa hay is unexcelled as a dry roughage for dairy cattle and is the standard with which other kinds of hay are compared."

A relatively new approach to hay harvesting involves addition of a small amount of chemical preservative to the hay at the time of baling. This permits baling at a higher moisture content than is otherwise recommended and in many instances reduces nutrient loss due to shattering of leaves during handling. This might also save millions of dollars that are lost annually due to spontaneous combustion of hay (Roethe, 1937). Research workers have reported hay quality to be inversely proportional to the moisture content of hay at baling and temperature attained during storage (Candlish et al., 1973; Gregory et al., 1963). It has been suggested by several workers that high temperature of hay during storage favors a chemical reaction in which hexoses and amino acids react to form indigestible components, thus lowering the feeding value of hay (Goering et al., 1973).

Various chemicals have been tested for effectiveness in preserving alfalfa hay. Those appearing to be of greatest value at present are ammonium isobutyrate, anhydrous ammonia, propionic acid and propionic acid combined with formalin (Barrington et al., 1975; Knapp et al., 1976; Sheaffer and Clark, 1975). The previous workers have indicated that the amount of these chemicals required to prevent heating and molding in baled hay is related to the moisture content of hay at baling and a higher level of application of chemicals is required as the moisture content of hay at baling is increased (Sheaffer and Clark, 1975).

No reliable data have been reported on the merits of adding organic acid preservatives to hay at baling for feeding to dairy cows. The objectives of this study were to: 1) compare the nutrient loss and resultant nutrient content of high moisture alfalfa hay with and without the addition of chemical preservative, with hay harvested in a conventional manner, and 2) evaluate the effect of adding the chemical preservative to alfalfa hay at baling on its feeding value for lactating cows.

CHAPTER II

LITERATURE REVIEW

Hay Quality Factors

Hay Moisture and Temperature in Storage

Miller et al. (1967) baled alfalfa hay at moisture content of 26.2, 35.2, 53.4 and 58.5% and native hay at 19.2, 34.1, 43.5 and 52.8%. They observed an increase in ash, acid detergent fiber and lignin as the moisture content of hay at the time of baling increased. There was little difference in protein or soluble carbohydrates, but the apparent digestibility of protein and energy decreased ($P<.01$) as moisture content at the time of baling increased. Gregory et al. (1963) reported that hay quality was inversely related to the initial moisture content of the stored product, and the temperature attained during storage. A decrease in dry matter digestibility in untreated hay and loss of dry matter in alfalfa hay stored for two months was related to the higher temperature reached during storage by Knapp et al. (1974). Baling hay at a moisture content greater than 20% resulted in forage of low quality.

Couchman (1959) determined the effect on clover-lucerne hay of storage at temperatures from -18 to 32 C for 8 to 9 months and reported that crude fiber content increased at temperatures of 21 to 32 C. The lignin content of stored hay was higher than of the unstored, but no change was observed in crude protein and ash content. Hays baled at 25% moisture had a temperature of 45 C, whereas that baled at an initial moisture content of 40% became very hot and reached a temperature of 60 to 65 C, with molding evident at both moisture levels. Gregory et al. (1963) studied mainly grass hays and suggested that the temperature increase was mainly caused by microbial action. The temperature increase indicated microbial oxidation and consequent dry matter destruction (Miller, 1947). Goering et al., (1973) compared the relative susceptibility of eleven forages (chopped, in jars), to heat damage as effected by moisture, temperature and pH, and observed heat damage at 60 C in a 24 hour heating period. The greatest amount of heat damage took place when moisture was more than 20% and there was a consistently higher acid detergent insoluble nitrogen in the ranges of 20 to 70% moisture when heating was for 48 hours. They further reported that large differences in susceptibility to heat damage appeared to be unrelated to species of hay, nitrogen or initial insoluble nitrogen content.

Roethe (1937) reported the results of a series of experiments which were conducted to observe the spontaneous heating and ignition of hay as related to its initial moisture contents. He reported that the maximum temperature produced in any experiment was 88 C, which was reached in alfalfa hay with moisture content of 50.5%. He further observed that maximum temperature was generally reached within 5 to 9 days after the hay was placed in storage. Moreover, hay of distinct brown color did not result if the moisture content of hay was not more than 30%. Currence and Searcy (1975) studied large fescue hay packages and reported lower digestibility of protein and dry matter in hay baled with higher initial moisture content as compared to well-cured hay. McKinley et al. (1976) reported similar results with grass hays. In contrast, Weeks et al. (1975) found that in vitro digestibility of alfalfa hay in loose stacks was not depressed by initial high moisture content indicating a potential for maintaining nutritive value of the loose-stacked hay with initial moisture contents as high as 40%.

Mohanty et al. (1967) studied the effect of molding on the feeding value and digestibility of alfalfa hay. They reported a temperature of 50 C during molding and average digestible dry matter and protein were higher for well-cured hay than for molded hay. The animals fed molded hay showed symptoms of depraved appetite. Gregory et al. (1963) observed losses of sugars and lipids in grass hays at higher moisture content.

Maillard Reaction (Hay Browning)

Goering et al. (1973) determined that heating period, temperature in storage and initial moisture content were related to the amount of browning that would result in hay. The maillard or the non-enzymatic browning reaction involves the condensation of carbohydrate degradation products with proteins or amino acids forming a dark colored insoluble polymer (Van Soest, 1965). Gregory et al. (1963), Hoffman (1940), and Hoffman and Bradshaw (1937) reported that high temperature during storage due to increased microbial activity would favor the maillard reaction in which hexoses and amino acid react to form indigestible compounds. Van Soest (1965) noted that non-enzymatic browning reaction required water. The sugar residues appeared to condense with amino groups at a 1:1 ratio. The extent of browning in heated forages is positively correlated with nitrogen bound in acid detergent fiber

(Gregory et al., 1963; Van Soest, 1962). The nitrogen bound in ADF had a low in vivo digestibility. The dark colored nitrogenous polymer resulting from the browning reaction was noted to accumulate in the lignin fraction of acid detergent fiber (Van Soest, 1965; Gregory et al., 1963).

Wang and Odell (1972) reported that pyrazines were obtained from heating certain aminohydroxy compounds such as ethanolamine, serine, threonine, etc., and these compounds were considered as one of the precursors of pyrazines in foods. The aroma of this class of compounds in foods has been described as "cooked" or "roasted" and their precursors have been considered as sugars and amino acids (Koehler and Odell, 1970). Koehler et al. (1971) determined the concentrations of alkylpyrazines in different foods. They observed that both the odor-detection threshold levels and a subjective evaluation of the odor of the compound coupled with the quantitative knowledge of pyrazine content of roasted food allowed an assessment of the probable significance of the pyrazines in the aroma of these products. Wang and Odell (1972) quantitatively investigated the volatiles in roasted pecans and separated into carbonyl, basic, acidic and non-carbonyl oxygenated fractions. Most of the carbonyls identified were unbranched aliphatic compounds and it is possible that they were the thermal degradation products of triglycerides. The compounds identified in basic fractions were alkylpyrazines. These pyrazines were considered to arise from the reaction intermediate of amino acids with sugars. They further observed that with the removal of carbonyl and basic compounds, the characteristic aroma of roasted pecans was lost. This binding of amino acids and sugars in foods due to the influence of higher temperatures seems similar to that which occurs in hay, although the resulting product and its significance may be different. This is only a part of the amadori rearrangement and browning reaction due to heat on foods.

Bechtel et al. (1945) suggested that alfalfa hay which contained 30% or more moisture when placed in stacks was likely to become brown during storage. This color varied from brown to black, depending on the amount of heat developed during storage. The browning process is associated with losses in organic matter and the feeding value is inversely proportional to the intensity of the brown color developed in hay. The percent losses in weight attributed to browning of legume hay were 19 for dry matter, 23 for NFE, 22 for CF and 9 for CP. The protein digestibility was affected most drastically, even on slight heating a 50% reduction in protein digestibility was noticed (Watson, 1939).

Dodd (1933) reported that stacked hay would retain some green color if no perceptible heat is present, but will appear light brown at 49 C, warm brown at 60 to 65 C and dark brown at 71 C. He further observed that sugars decomposed into glucic acid and unsaturated compounds which unite with oxygen to produce additional heat. Gordon et al. (1961) compared the preservation and feeding value of alfalfa stored as hay, haylage and silage and observed highest feeding values, animal acceptance, milk production and digestibility coefficients for barn dried hay. Yu (1977) heated dried alfalfa leaves, stems and hay at nine different temperatures (45 to 170 C) and for different durations (8.9 to 120 hours). He observed that acid detergent-N was affected in dry samples when maximum temperatures exceeded 100 C and in wet samples $\leq$95 C affected recovery of acid detergent insoluble nitrogen. Density of hay package affects temperature rise and spontaneous heating in high moisture bales. Raising the density of hay bales increases temperatures reached during storage of alfalfa hay. High density did not allow heat to escape from the hay package causing a more elevated temperature. This was true especially when hay was put in large, dense hay packages (Nelson, 1966).

Organic Acid Preservation

Although several chemicals have been identified as having fungistatic and fungicidal properties, there is a paucity of data directly comparing their merits and no single chemical or combination of chemicals has emerged as the material of choice for a particular preservation requirement. Organic acids have been repeatedly tested for their efficacy in preserving high moisture grains and silages for the last several years. Stevenson and Alexander (1972) successfully used propionic acid at .75% level to preserve high moisture (18 to 22%) soybeans. Addition of organic acids to preserve hay is a relatively new approach in hay harvesting. Storage of high moisture hay up to 40% would be possible if mold growth were inhibited (Candlish et al., 1975). Stevenson (1972) reported that propionic acid had strong fungicidal and fungistatic properties. Mohanty et al. (1967) observed that mold growth was primarily responsible for heating of hay and caused further deterioration.

Goering and Gordon (1973) reported that many problems of efficient feed storage are directly or indirectly related to growth of mold when forages are stored between 25 and 55% moisture. In three trials they evaluated effectiveness of several chemicals as mold inhibitors for forage storage. Chemicals tested were sodium chloride, sodium propionate, 57:40 acetic and propionic acid combination and ammonium isobutyrate at .2, .4, .6, .8 and 1% levels. The trial results were varied but propionic acid was found to be the most effective mold inhibitor on chopped alfalfa hay. In another trial effectiveness of propionic acid, acetic:propionic acids in ratio of 57:40 and 1.1% ammonium isobutyrate for preventing molding and healing was tested. Ammonium isobutyrate and acetic:propionic produced lower temperatures than other treatments, however, propionic acid at .8 or 1% prevented temperatures from going as high as controls. In the third trial, ammonium isobutyrate was found to be an effective mold inhibitor. Knapp et al. (1976) added propionic acid to alfalfa hay (32.4% moisture) just after baling at levels .02, .2, .5 and 1.0% of weight of hay as preservative. They reported that all of the untreated hays became molded, heated to more than 55 C and showed significant losses in dry matter, soluble carbohydrate and had lower *in vitro* dry matter disappearance. They observed that degree of visible molding in hays was lowered by increasing the application rate of propionic acid, and propionic acid at 1% level effectively prevented heating. Candlish et al. (1975) studied the effects of commercial hay preservatives containing 20% propionic acid, at levels .05 to .11% on orchard grass, clover and alfalfa-brome mixture baled at 40% moisture. They reported a temperature of 58.3 C in untreated orchard grass and visible mold after 3 days, whereas no visible mold was seen in preservative treated orchard grass bales which reached a temperature up to 33 C. Sheaffer and Clark (1975) treated alfalfa-timothy hay at 31% and 40% moisture with propionic acid at levels of 1.0, 1.5 and 2% at the time of baling. They suggested that an increased level of acid treatment be used as moisture content of hay increased. A significantly lower temperature during storage was observed in acid preserved hay as well as higher *in vitro* dry matter digestibilities compared to untreated hay or hay treated with 1% level of acid. They further suggested that to preserve hay at 40% moisture, 3 to 5% propionic acid application was needed. Knapp et al. (1974) reported that 1% level of propionic acid or anhydrous ammonia application to alfalfa hay with 35.0% moisture lost only about half as much digestible dry matter as untreated hay, or hay treated with less than 1% propionic acid. They applied anhydrous ammonia at 1% level and propionic acid at .02, .2, .5 and 1% level as hay preservative to hay baled at 35% moisture content. After two months storage the workers observed that anhydrous ammonia treated hay was green with no visible molds but total nitrogen content of hay was increased by 30% due to ammonia treatment. It was further observed that hay treated with 1% propionic acid was also without visible mold but had lost green color. However, both treatments prevented hay temperature during storage from going as high as in controls. Jorgenson et al. (1973) reported a higher percent of total nitrogen in the ADF-N fraction in alfalfa hay baled at 25 to 37% moisture with the addition of 0.5% propionic acid or 1% propionic acid plus .5 g formaldehyde/100 g crude protein than the hay baled at 16 to 19% moisture. The quality and digestibility of alfalfa hay baled at 29 or 26% moisture with the addition of .1% commercial mold inhibitor containing 20% propionic acid as active ingredients, was similar to the hay baled at 11 or 16% moisture (Asplauud, 1971).

Barrington et al. (1975) applied an acid preservative compound at .1% level which had 20% propionic acid as active ingredient, in three successive trials to alfalfa hay that ranged from 25 to 37% moisture content. The control hay was baled at 16 to 20% moisture content. The ADF-nitrogen values for the treated hays were significantly higher than the control hay indicating a heat damage to wet hays. They suggested that the commercial product at the recommended level did not effectively control the hay temperature during storage and thus dry matter loss resulted. Barrington et al. (1975) conducted other trials, in which they applied propionic acid at .5 and 1% levels, 1% and propionic acid plus .055% formaldehyde. They reported excellent control of dry matter losses with both 1% propionic acid and 1% propionic acid plus .055% formaldehyde.

Goering et al. (1973) studied the effect of propionic, propionic: acetic acid mixture and ammonium isobutyrate as mold inhibitors on a grass-clover mixture and observed that ammonium isobutyrate was the most effective mold inhibitor. However, Knapp et al. (1974) suggested that further research must be conducted to determine whether there were any detrimental effects from long term feeding of hay preserved with ammonia or propionic acid. Although they suggested that anhydrous ammonia and propionic acid could be used to control temperature of hay stored with higher moisture content, it was suggested that the optimum rates and frequency of application should be determined by further research. Mueller et al. (1976) applied 1 to 2% level of ammonium isobutyrate to high moisture forages (>25% moisture) and reported that its preservative effect rendered forage equal to the quality of artificially dried hay. McNemar et al. (1977) found that storage temperature was reduced when ammonium isobutyrate was applied at levels of 1.5%. In a preliminary experiment, Knapp et al. (1974) treated 300 kg of baled hay with 52% moisture (which was heated to 63 C) with 5 kilograms of anhydrous ammonia. They observed that temperature of the hay dropped to 35 C within 48 hours in contrast to control bales that maintained high temperatures for 2 weeks. They suggested that anhydrous ammonia may protect against the danger of spontaneous combustion. Yu and Thomas (1975) reported that adjusted means for total solids and fat in milk of cows were higher ($P<.05$) when fed haylage sprayed with .8% propionic acid as compared to that of those fed haylage with lower rates of acid applications. They concluded that higher rates of application of propionic acid or ammonium isobutyrate on alfalfa haylage would result in higher milk solids and milk fat than lower rates.

Significance of Oven-Temperature

Several workers have criticized the usual sample drying technique in the oven at 100 C for 24 hours for dry matter determination and for preparation of samples for other analyses. However, no solid information is available to be used as a correction factor if a sample is dried at 100 C for 24 hours. Van Soest (1965) noted that heat drying forage above 50 C significantly increased fiber and lignin. The increased yield of ADF could be accounted largely by the production of artifact lignin via a non-enzymatic browning reaction. Van Soest (1965 (1965) and Goering et al. (1972) suggested that at higher temperatures under certain conditions there could be a serious effect on analytical results and a marked decline on <u>in vivo</u> digestibility. In contrast, Tilley and Terry (1963) who devised a two-stage technique for <u>in vitro</u> digestion of forage crops have prescribed drying samples for analysis at 100 C for 6 hours. Hesse and Kennedy (1956) recommended that samples should be oven dried at temperatures between 70 to 95 C cause at this temperature range, the chemical composition of wet grain samples (oven dried) was observed to be similar when expressed on dry matter basis. They further suggested that for both speed and accuracy, in forage research it was necessary to make many individual measurements of dry matter contents of forage. Van Soest (1965) suggested that mere addition of protein to a lignin determination does not increase the lignin value but it required that the protein be bound to fiber through the maillard reaction or precipitation with tannin. The maillard reaction is enhanced by moisture contents in the range of 20 to 50%, but was not significant in dry samples heated at higher temperatures. Temperatures of 55 to 60 C was enough for safe oven drying. The maillard reaction product was found resistant to proteolytive enzymes, hence the preliminary drying of samples at 100 C might not be practiced.

In Vitro Dry Matter Digestibility (IVDMD)

"It is not surprising that *in vitro* rumen procedures, particularly that of Tilley and Terry (1963), yield more accurate estimates of digestibility than do chemical methods" (Oh et al., 1966). This superiority of *in vitro* techniques should be expected because the digesting bacteria are sensitive to undetermined factors influencing the extent of digestion (Van Soest, 1967).

Knapp et al. (1976) observed a decline in *in vitro* dry matter disappearance in hay (70.5% to 60.5%) from the time of baling to after storage for 2 months without any treatment (P<.05). They further reported that IVDMD of hay treated with 1% propionic acid was about 4.5% higher than control hay (P<.05). The decline in IVDMD in wet heated heated hay was attributed to loss of highly digestible cellular contents such as carbohydrates and lipids (Watson and Nash, 1960). Of the procedures used in *in vitro* DMD studies conducted by Yu and Thomas (1976), the most complicated was that using rumen microbial fermentation plus pepsin as discussed by Goering and Van Soest (1970). *In vitro* dry matter or organic matter digestibility of small samples may be used to evaluate the *in vivo* digestibility of grasses and forages

(Tilley and Terry, 1963). Donefer (1963) reported the usefulness of *in vitro* technique in predicting forage nutritive value that was positively related to the nutritive value as measured in *in vivo* trials. He noted that the major source of error in experimental studies was due to the difference in chemical composition of forages caused by lack of uniformity of test material. Sheaffer and Clark (1975) reported that hay sprayed with 1.5 to 2.0% propionic acid or ammonium isobutyrate had significantly higher IVDMD than untreated alfalfa-timothy hay which showed higher ADF content.

Rumen VFA Composition

Stevenson (1972) used propionic acid to preserve wet grains and reported that the propionic acid treatment kept feeding value of grain at least equal to fresh untreated grains and was not toxic to animals. The effect of intraruminal infusion of VFA's and lactic acid on dairy cattle voluntary intake was determined by Montgomery et al. (1963). They reported a significant daily intake decrease by acetic acid and butyric acid infusion, but 280 g propionic acid infusion did not result in a significant decrease in hay consumption. This suggested that a propionic acid treatment to hay will in no way effect level of voluntary intakes.

CHAPTER III

CHEMICAL PRESERVATION OF ALFALFA HAY FOR DAIRY COWS

Summary

Alfalfa hay from a common field was baled at an average moisture content of 19% (dry control), at 28% with the addition of a chemical preservative, and at 29% with no preservative (wet control). The preservative consisted of 70% propionic acid and 30% formalin. Heating in storage and molding were prevented by the chemical treatment. After four months of storage, the hay baled at 29% moisture content without a preservative had higher acid detergent fiber (ADF) and ADF-N content than hay baled dry or with a preservative. The ADF-N and ADF-N as percent of total nitrogen values were significantly higher ($P<.05$) in treated and dry control hays dried at 100 C in comparison to 50 C drying. There was a trend for lower *in vitro* dry matter digestibility (IVDMD) of wet baled hay, though the treatment X hour, and treatment X month interactions did not allow clear interpretation of results for IVDMD. Yield and composition of milk from 16 lactating Holstein cows fed the dry control and the treated hays in a switchback trial were similar. The digestibility of dry matter, total protein, neutral detergent fiber, and organic matter were also similar in dry and treated hays. Molar percentages of rumen VFA's were similar in the two groups.

Applied at 1% of the bale weight, the preservative was effective in maintaining hay quality similar to that of hay baled at 19% moisture content.

Introduction

Hay quality is inversely related to initial moisture content and temperature during storage (Gregory et al., 1963). Increased microbial activity and heating result in greater losses of dry matter, sugars, fats and hemicellulose (Hoffman and Bradshaw, 1937). High temperatures during hay curing favor the maillard or browning reaction, in which hexoses and amino acids react to form indigestible compounds (Gregory et al., 1963; Nisson, 1963; Van Soest, 1965). Van Soest (1962) noted that the extent of browning in heated forages was directly correlated with the nitrogen bound in acid detergent fiber (ADF) which had low _in vivo_ digestibility. Goering and Gordon (1973) observed that the effective heating period, temperature and moisture content were related to the amount of browning in forages. Moreover, decreased utilization of protein in heated alfalfa hay by dairy cows has been observed (Bechtel et al., 1945).

Various chemicals have been listed as effective preservatives for alfalfa hay baled at high moisture content (Barrington et al., 1975; Knapp et al., 1974, 1976; Sheaffer and Clark, 1975), including propionic acid, anhydrous ammonia, ammonium isobutyrate and propionic acid combined with formalin. No reliable data have been reported on the merits of adding chemical preservatives to hay at baling for feeding to dairy cows.

The objectives of this study were to: a) compare the nutrient loss and resultant nutrient content of high moisture alfalfa hay (with and without chemical preservative), with hay harvested in a conventional manner and b) evaluate the effect of adding chemical preservative to alfalfa hay at baling on its feeding value for lactating cows.

Materials and Methods

Mid-bloom alfalfa hay from a common field was baled as follows: a) dry control, with average moisture content of 19%, b) wet control, at 29% moisture content and c) at 28% moisture with the addition of a commercial hay preservative[2] consisting of 70% propionic acid and 30% formalin. Rate of application of active ingredient was 1% of the hay as baled. The preservative was diluted 50:50 with water before application to permit more uniform distribution on the hay. This also equalized the moisture content of treated hay and the wet baled hay. The chemical was applied with a positive pressure spray unit (Figure 1) similar to that of McNemar et al. (1977) as the hay was picked up from the windrow and as it moved into the baling chamber.

Temperatures in a 30-bale stack of each type were monitored during the first 3 wk of storage, using thermocouples placed near the top and bottom parts of the stacks. Visual observations for evidence of molding were made after the hay had been in storage for 4 months. To determine chemical composition of the hay after storage, individual samples from six bales of each type were taken after four months and analyzed for total protein according to AOAC (1965), ADF and lignified nitrogen (ADF-N) according to the methods of Van Soest (1962) and Van

[2]Chemstor[R], produced by Celanese Chemical Company, Corpus Christi, TX.

Figure 1. Spray Unit in Operation.

Soest and Wine (1967). Prior to these analyses, drying was at 50 C for 72 hr. Drying of samples for dry matter determination and preparation for other analyses was at 100 C for 24 hr. To determine any adverse effect of oven drying of cured, air-dry hay in a conventional manner (100 C for 24 hr) individual samples from six bales of each type, were split into two: one-half dried at 100 C for 24 hr and the other half at 50 C for 72 hr. The effects of the two different drying temperatures on ADF-N, ADF, total protein (TP) and ADF-N as percent of total nitrogen components of hay were determined.

Seven bales of each type of the hay were sampled individually for 4 months at monthly intervals and IVDMD was determined following a modification of the procedure of Tilley and Terry (1963) for IVDMD.

Sixteen lactating Holstein cows were used in a feeding trial to compare the chemically treated hay with dry control hay. Cows were assigned to treatments at 7 to 10 wk postpartum following a preliminary feeding period. A switchback design (Brandt, 1938; Lucas, 1956) with 5 wk periods was used (Table I). Production data from the last 4 wk of each period were used for comparing treatments. Alfalfa hay comprised one-half of the total ration and the other half was concentrate mix (Table II). Initial feed allowances to meet 1971 NRC requirements were on the basis of weight and age of cow, milk yield and milk fat percent. These allowances were reduced by 5% at the start of each subsequent period to minimize weight changes during the trial.

Milk production was recorded twice daily with samples from four consecutive milkings each week and composited for analysis of total solids and milk fat percentages. Body weights of cows were recorded on three consecutive days at the beginning of the trial and at the end of

TABLE I

EXPERIMENTAL DESIGN

Experimental Period	Treatment Sequence	
	A	B
1	1[a]	2[b]
2	2	1
3	1	2

[a]Ration containing control hay.

[b]Ration containing treated hay.

TABLE II

CONCENTRATE MIXTURE

Ingredients	As-fed Group I	As-fed Group II
	------%-------	-------%-------
Wheat Middlings	50.00	----
Corn	27.50	50.00
Sorghum Grain (Milo)	----	17.50
Soybean Meal	12.50	22.50
Molasses Liquid	8.00	7.50
Dicalcium Phosphate	1.50	2.00
Salt	0.50	0.50

Group I - First 8 cows in experiment.

Group II - Last 8 cows in experiment.

each comparison period. Digestibility of ration components was determined during the fifth week of each period, using chromic oxide as an indicator. Chromium analysis was by atomic absorption spectrophotometry (Williams et al., 1962). Adjustment for diurnal variation in chromic oxide excretion was based on samples at 4 hr intervals for 2 days from six randomly selected cows in each period. Total and individual concentrations of rumen volatile fatty acids (VFA) were determined according to Erwin et al. (1961) on rumen fluid collected from each cow 3 hr after feeding during the last week of each period.

Results and Discussion

Temperatures in the stack of wet baled hay during the first 10 days of storage were considerably higher than in the treated and dry hay stacks (Figure 2). Temperatures observed in the thirty-bale stack of chemically treated hay were similar to those in a considerably larger stack of hay of the same type stored in another location. No visible mold was observed in the hay treated with chemical preservative or in dry baled hay after 4 months of storage, whereas the wet baled hay was extensively molded (Figure 3). Addition of the propionic acid-formalin in mixture at 1% of the hay as baled effectively controlled heating and mold growth during storage. Similar results were obtained by Barrington et al. (1975) with a slightly higher rate of chemical application on alfalfa hay with 37% moisture at baling. Knapp et al. (1974, 1976) found application of 1% of either propionic acid or anhydrous ammonia effective in controlling heating and molding in alfalfa hay baled at 32 and 35% moisture, whereas Sheaffer and Clark (1975) found 1.5% propionic acid or ammonium isobutyrate necessary for effec-

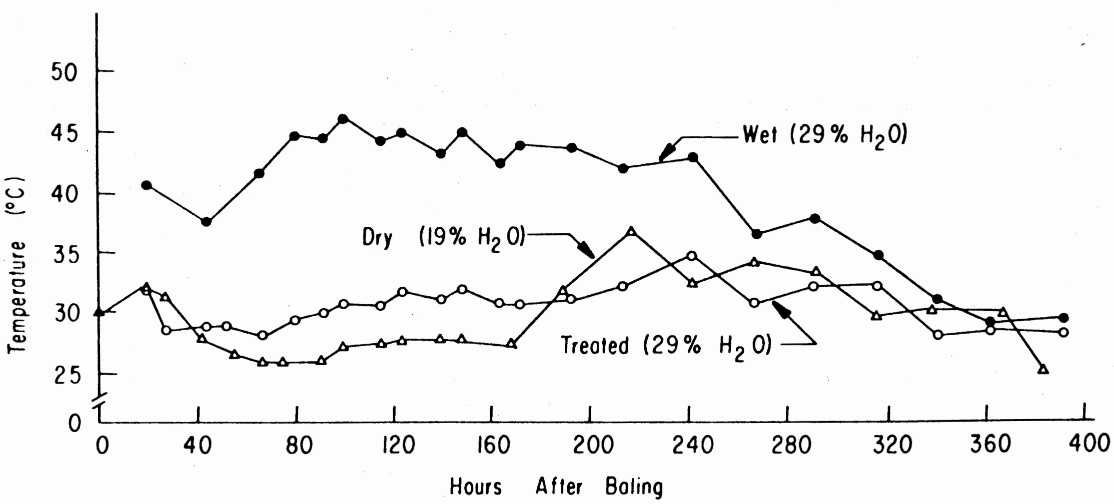

Figure 2. Temperature of Hay in Storage.

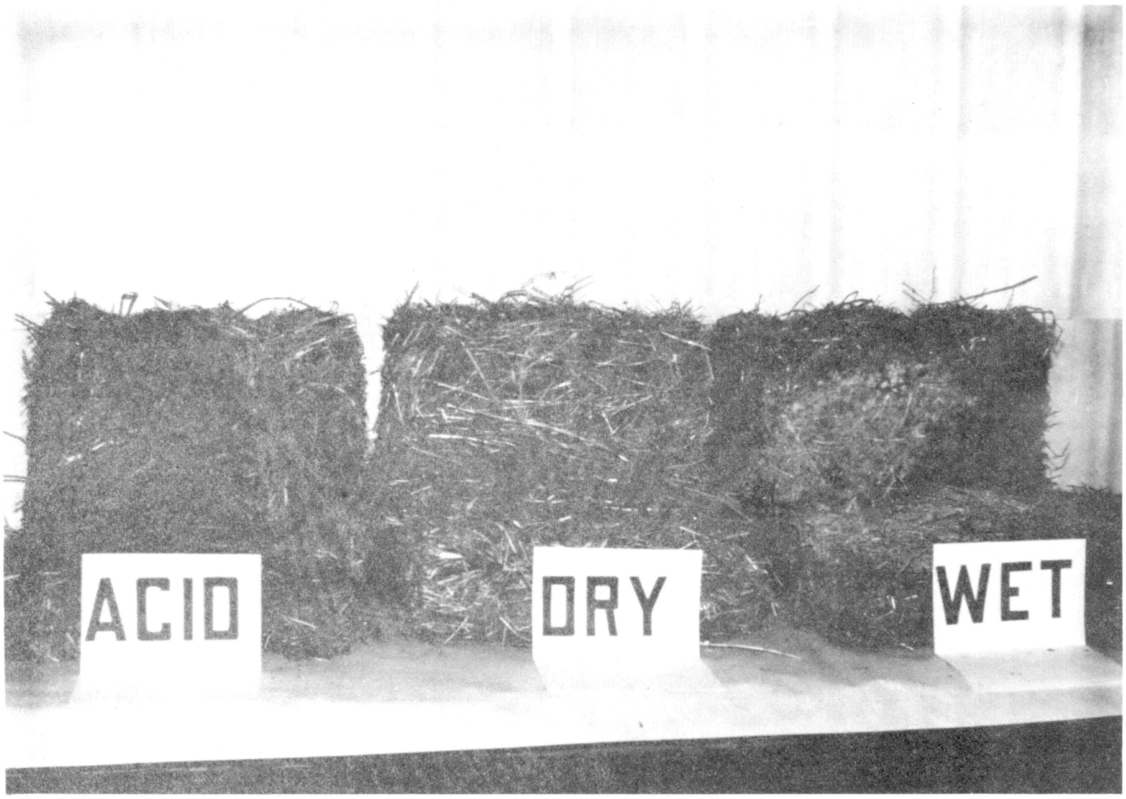

Figure 3. Molding of Hay in Storage.

tive preservation of alfalfa-timothy hay baled at 31% moisture content.

After four months of storage, the hay baled at 29% moisture content without a preservative had higher ADF and ADF-N content than hay baled at 19% moisture content or at 28% moisture with a preservative added (Table III). Presumably, the increase in ADF-N content of the wet baled hay was caused by the browning reaction during heating (Nissen, 1963). Increased ADF content may be attributed to loss of more soluble carbohydrates in hay subjected to heating (Knapp et al., 1976). In any event, addition of the propionic acid-formalin preservative to hay baled at high moisture content was effective in maintaining its composition similar to that of dry baled hay.

Due to a significant ($P<.02$) interaction between the treatments and temperatures of drying, the overall effect of higher temperature on ADF-N, acid detergent fiber, total protein and ADF-N as percent of total nitrogen could not be interpreted. However, when the effect of different oven temperatures within each treatment were determined, the ADF-N, and ADF-N as percent of total nitrogen in treated and dry control hays and ADF component in dry control hay were observed to be significantly higher ($P<.03$) for the samples dried at 100 C (Figure 4, 5, 6) in comparison to the ones dried at 50 C. These findings were in agreement with the results of Van Soest (1965) who reported that increased drying temperature would increase the quantity of insoluble protein. The two drying temperatures for the wet control hay did not produce any significant differences in any of the above components (Figure 4, 5, 6). This indicated that drying the treated and dry hays at the higher temperatures increased the percentage of lignified nitrogen as compared to drying at lower temperatures (Figure 4, 5). Van

TABLE III

COMPOSITION OF HAY AFTER STORAGE FOR FOUR MONTHS

Item	Hay Treatment			SE
	Preservative Added	Dry Control	Wet Control	
	-----------------%-----------------			
H_2O Content at Baling	28	19	29	---
Total Protein	14.5	15.4	15.0	.80
ADF	38.6[a]	35.8[a]	43.2[b]	.91
ADF-N	.21[c]	.21[c]	.29[d]	.03
ADF-N/Total N	8.9[a]	8.3[a]	12.0[b]	.62

[ab]Means with different letters significantly different (P<.01).

[cd]Means with different letters significantly different (P<.05).

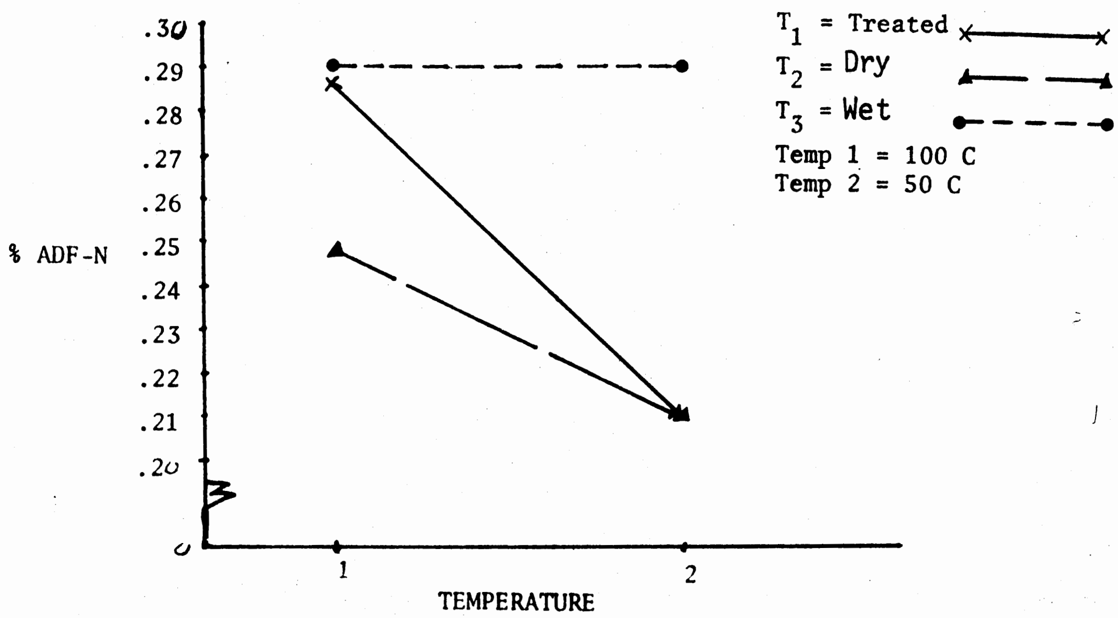

Figure 4. Percent ADF-N in Hay Dried at Different Oven Temperatures.

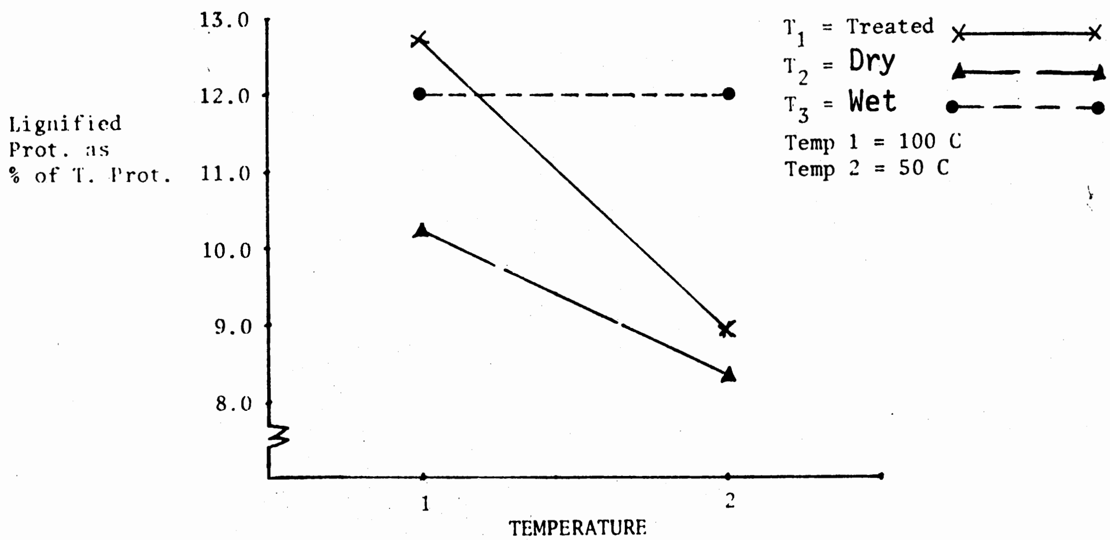

Figure 5. Lignified Protein (ADF-N) as Percent of Total Nitrogen in Hay Dried at Different Oven Temperatures.

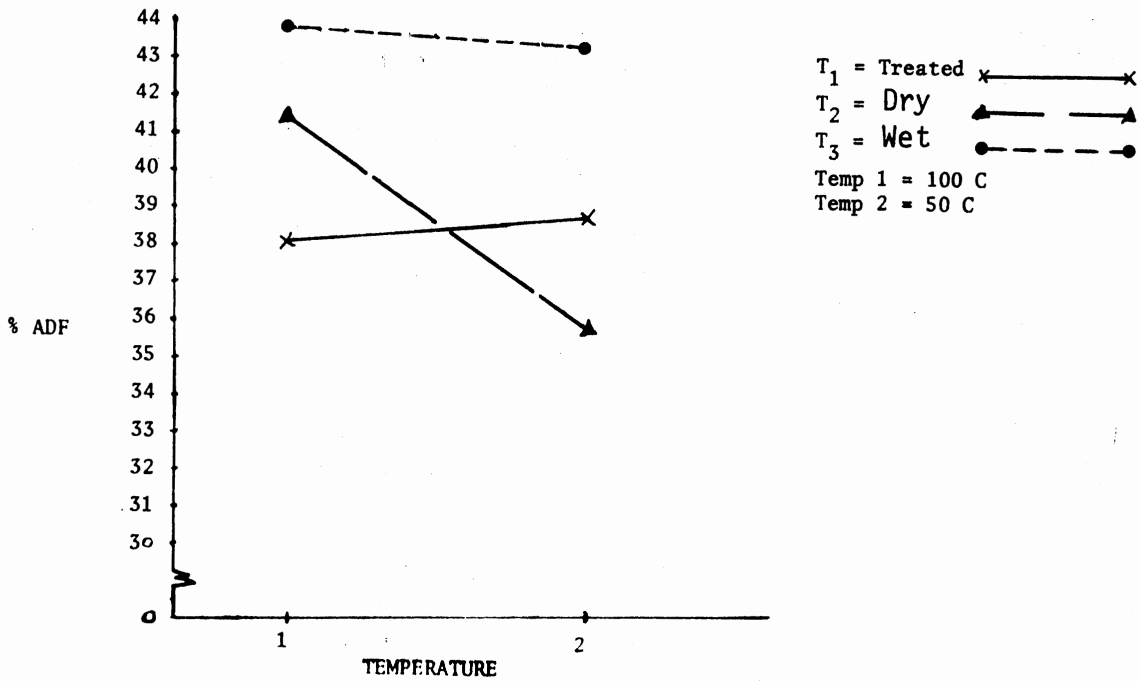

Figure 6. Percent ADF in Hay Dried at Different Oven Temperatures.

Soest (1965) observed smaller increases in ADF and ADF-N of dry baled hay than in hay baled at high moisture content. In a similar study, Yu (1977) reported that quantitative changes of acid detergent insoluble nitrogen in dry samples were effected by temperature exceeding 100 C, but in wet hay $\leq$95 C was enough to increase recovery of acid detergent insoluble nitrogen. The total protein values for all the three treatments were similar at both temperatures (Figure 7).

Treatment X hour interaction in regard to IVDMD was expected due to the manner in which the test was performed (Figure 8), as the bacteria in the inocculum could respond differently on different hay depending on time available for digestion, number of bacteria and digestibility of hay. There appears to be a trend for lower IVDMD of wet baled hay (Figure 9); however, the treatment X month interactions made it impossible to conclude anything definite about the digestibility. For example, at 18 hr, month 3 and 36 hr, month 4 (Figure 10, 11), the wet baled hay had higher IVDMD than acid treated hay. This shows that months were confounded with some unknown factor, that could be due to different rumen inocculum used for each month, sampling error or undetermined procedural error.

In the feeding trial, comparison was made only between the hay baled dry and that treated with preservative. Both were readily accepted by the cows resulting in no difference in average intake under the conditions of this trial (Table IV). Similarly, Tabor (1977) reported equal intake of dry baled hay and hay treated with .1% chemical preservative containing 20% propionic acid. Average milk yield of cows fed chemically treated hay was slightly higher than that of the cows fed dry baled hay (Table IV); however, the differences were not stat-

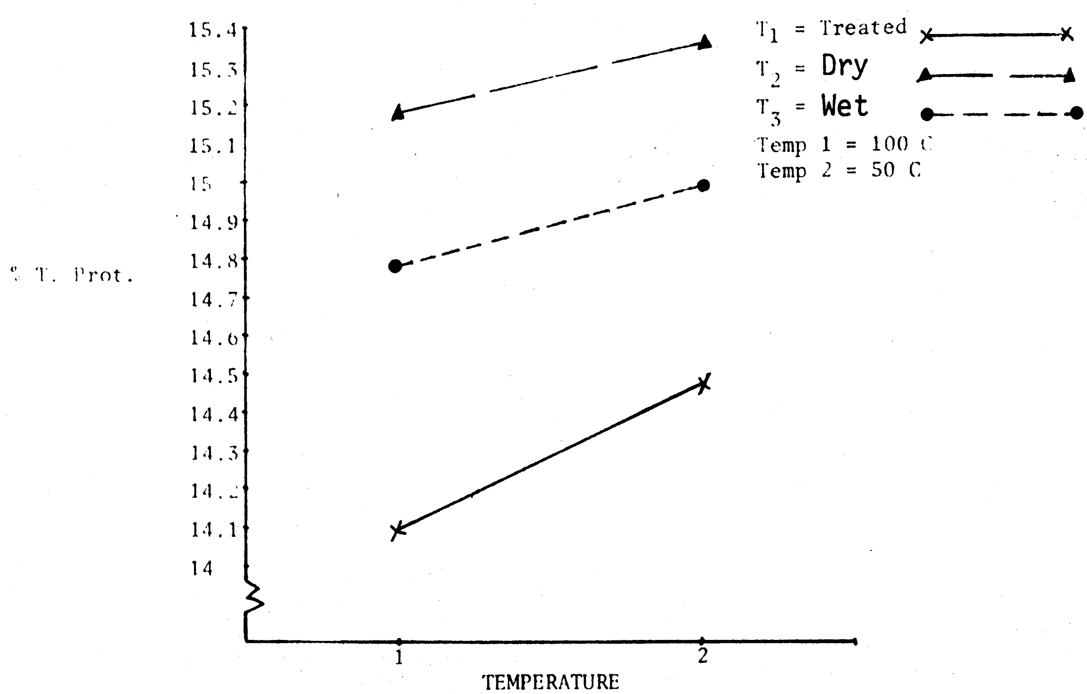

Figure 7. Percent Total Protein in Hay Dried at Different Oven Temperatures.

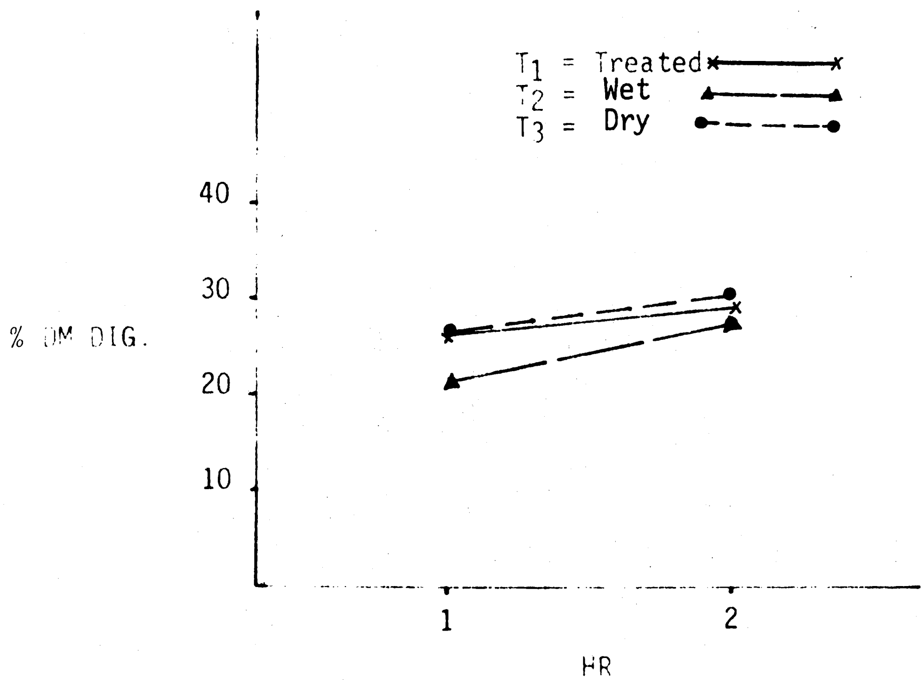

Figure 8. Percent IVDMD, Four Months Averaged.

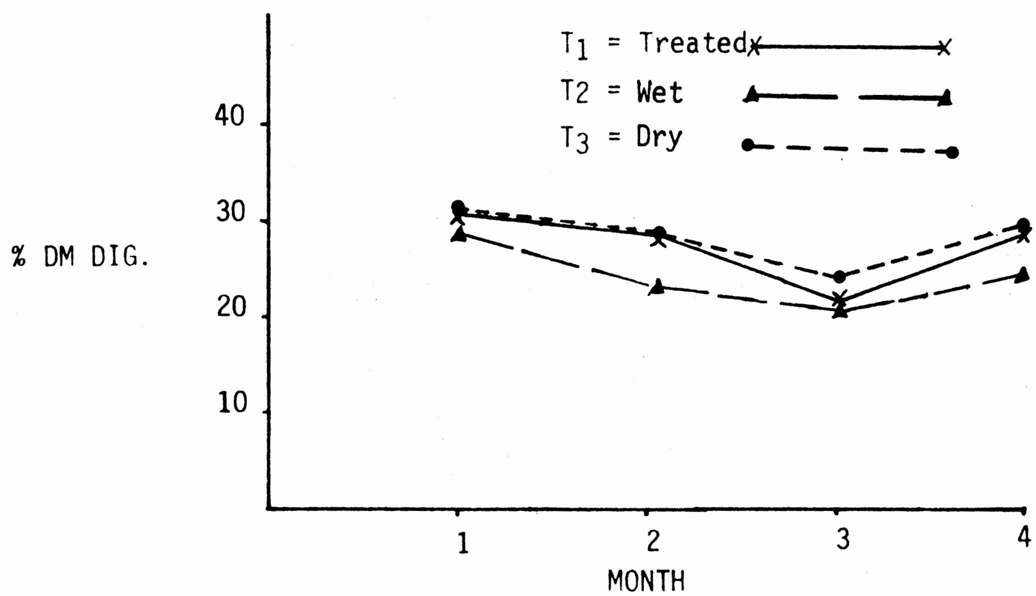

Figure 9. IVDMD, Hour 18 and 36 Averaged.

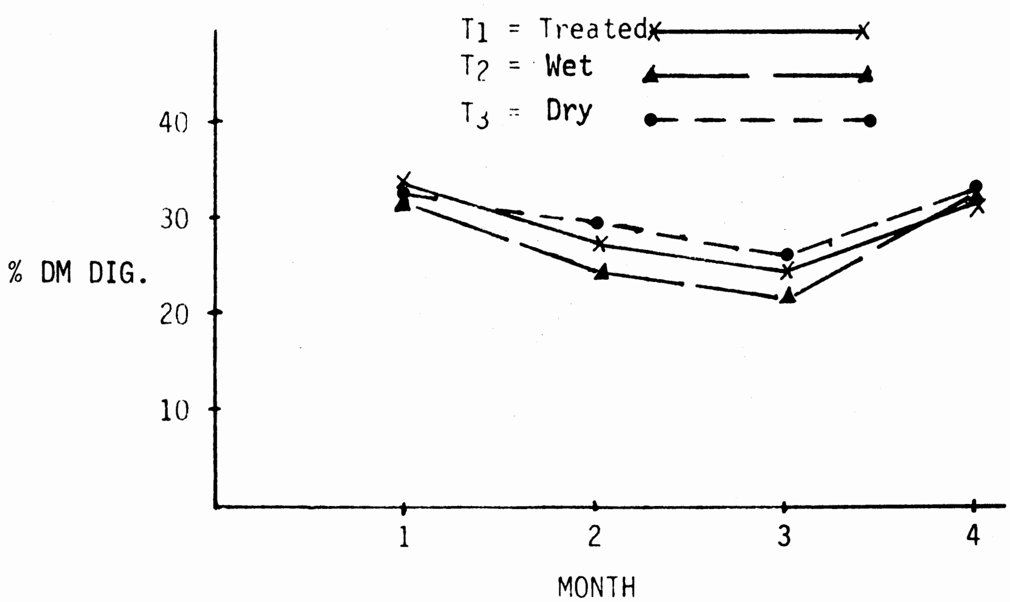

Figure 10. Percent IVDMD After 36 Hour of Incubation.

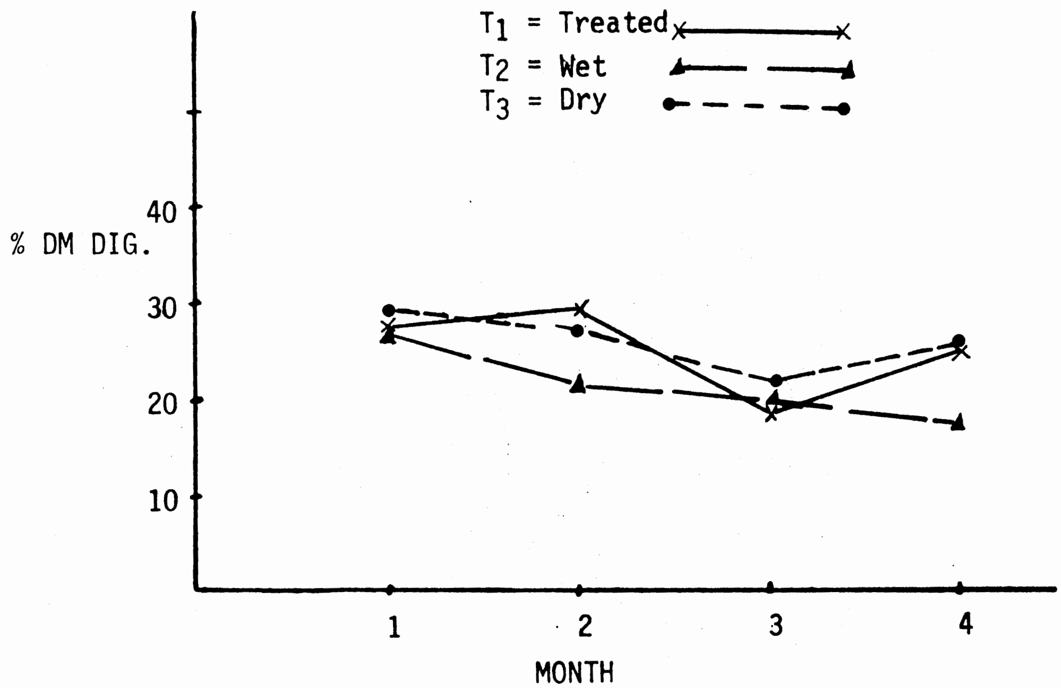

Figure 11. Percent IVDMD After 18 Hour of Incubation.

TABLE IV

RESPONSES OF COWS IN FEEDING TRIAL

Item	Hay Treatment		SE
	Dry Control	Treated	
Feed Intake			
Grain, kg/day	9.6	9.7	.04
Hay, kg/day	9.6	9.6	.04
Milk Production			
Yield, kg/day	24.7	25.0	.14
Fat, %	3.68	3.61	.03
SNF, %	8.56	8.55	.05

istically significant (P>.09). In contrast, Tabor (1977) observed that cows fed alfalfa hay baled at about 28% with addition of .1% commercial preservative produced significantly less milk than cows fed hay baled at 20% moisture content, which might be due to lower level of acid application. Milk fat and non-fat solid percentages were similar for cows fed the two kinds of hay (Table IV).

Average percent digestibilities of total ration dry matter, NDF, crude protein and organic matter were similar for cows fed the chemically treated and control hays (Table V). These digestibility values for the hay components were in agreement with the production data (Table IV) and supported the conclusion that the chemically treated hay was at least equal in its feeding value to the dry control hay for lactating cows.

The molar percentages of rumen VFA's in cows fed treated and dry control hay were similar (Table VI). The amount of propionic acid added to the hay at baling was not reflected in significantly higher rumen concentration of propionic acid and did not affect milk fat percentage.

The chemical preservative applied at 1% of alfalfa hay averaging 28% moisture at baling was effective in maintaining hay quality. The feeding value of this chemically treated hay was at least equal to that of dry baled hay.

Economic Value

The economic merit of adding a chemical preservative depends on several factors. Holter and Young (1978) suggested that the value of hay additives appeared to be in salvaging hay that is nearly dry (less

TABLE V

DIGESTIBILITY OF RATION COMPONENTS

Component	Treatment Group Dry Control	Treatment Group Treated	SE
	------------%-----------		
Dry Matter	59.2	60.4	.81
Total Protein	61.5	61.7	.81
NDF	57.1	57.6	1.18
Organic Matter	62.7	63.4	.83

TABLE VI

MOLAR PERCENTAGES OF RUMEN VFA

Acid	Treatment Group		SE
	Dry Control	Treated	
Acetic	64.04	65.10	1.02
Propionic	19.04	18.24	.36
Butyric	11.42	11.70	.89
Isobutyric	1.77	1.55	.12
Valeric	1.60	1.50	.15
Isovaleric	2.12	1.92	.20

than 25% moisture) rather than any positive effect on feed intake or nutritive value. It is evident from our findings of the present study, that 1% of the commercial preservative applied to hay at 28% moisture successfully prevented heating and molding during storage and its feeding value was similar to the hay baled at 19% moisture. However, for successful results the preservative should be applied as evenly as possible (Mueller et al., 1976).

The cost of chemical plus the initial cost of the applicator will increase the cost of hay, depending on the preservative and the apparatus used. For example, Chemstor^R cost \$8.80 per metric ton when applied at 1% level which seems to be the adequate rate of chemical application as observed from the present study. An applicator costs \$400.00 or more with additional labor being required to calibrate the spray apparatus and to handle heavier hay. The organic acids are corrosive which increase wear on the hay baling equipment. Precautions must be observed when handling preservatives.

The farmer has to balance the cost of preservative and the weather damage or the complete loss. From the results of this study it appears that chemical preservation of alfalfa hay with good quality preservative at 1% level of application will reduce field losses and allow hay to be baled at higher moisture levels. A dairy farmer or the hay buyer should consider the moisture difference in buying the hay baled at conventional moisture levels and at relatively higher moisture containing hay treated with chemical preservative. For example, a metric ton (1000 kg) of hay baled at 19% moisture would contain 810.0 kg of dry matter whereas a ton of hay at 28% moisture would contain 720.0 kg of dry matter. Therefore, the price paid for wet hay should be about 89% of the dry hay price. If the price of the hay were $75 per metric ton the wet hay would be worth $66.75 per metric ton ($75 X .89 = $66.75).

However, at this point it might be kept in mind that the chemical treatment is a convenient management tool to prevent the losses due to inclement weather and allows proper storage under farm conditions and should not be expected to improve the efficiency of feed utilization. Production level may not be effected by the concentration of preservative used in this study.

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APPENDIX

TABLE V

ANALYSIS OF VARIANCE FOR FEEDING TRIALS

Source of Variance	Degree of Freedom
Linear Term	1
Quadratic Term	1
Pair	7
Sequence	1
Pair X Sequence	7
Linear Term X Sequence	1
Linear Term X Pair	7
Linear Term X Pair X Sequence	7
Quadratic Term X Pair	7
Quadratic Term X Sequence[a]	1
Quadratic Term X Pair X Sequence[b]	7
Total	47

[a]Quadratic Term X Sequence interaction is equal to the effect due to treatment.

[b]Quadratic Term X Pair X Sequence interaction was the error term used to test the significance of treatment effects.

TABLE VI

TEST OF SIGNIFICANCE FOR COW TRIAL

Item	Treatment M.S.[a]	Error M.S.[b]	Probability
Milk	1.59	.442	.099
Milk Fat	.052	.014	.096
Non-fat Solids	.0015	.058	.88
Solids Corrected Milk	.118	.434	.62
Dry Matter Digestibility	16.63	13.908	.31
Crude Protein Digestibility	.858	14.138	.81
Neutral Detergent Fiber	3.197	29.487	.75
Organic Matter Digestibility	5.145	14.464	.57
Hay Intake	.001	.036	.87
Grain Intake	.026	.036	.42

[a]The effect due to treatment is equal to the interaction of the quadratic and sequence group (a fraction of the sum of squares of the D value totals).

[b]The error term is a pooled interaction between the quadratic term and individuals in the two groups (PQ X Pair X Seq.).

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