
GHUMAIZ
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N. A. AL-GHUMAIZ

M.S

2003

This is to certify that the

thesis entitled

EFFECT OF MORNING VERSUS AFTERNOON CUTTING TIME ON ALFALFA SUGAR CONTENT AND SILAGE ACID PROFILE

presented by

Nasser S. Al-Ghumaiz

has been accepted towards fulfillment
of the requirements for

Master of Science degree in Crop and Soil Sciences

Major professor

Date 7/25/03

EFFECT OF MORNING VERSUS AFTERNOON CUTTING TIME ON ALFALFA SUGAR CONTENT AND SILAGE ACID PROFILE

By

Nasser S. Al-Ghumaiz

A THESIS

Submitted to
Michigan State University
In partial fulfillment of the requirements
For the degree of

MASTER OF SCIENCE

Department of Crop and Soil Sciences

2003

ABSTRACT

EFFECT OF MORNING VERSUS AFTERNOON CUTTING TIME ON ALFALFA (*Medicago sativa*) SUGAR CONTENT AND SILAGE ACID PROFILE

By

Nasser S. AL-Ghumaiz

The assessment of cutting time of morning versus afternoon has not been studied on alfalfa in the Great lakes region. This study was conducted to evaluate the effect of morning versus afternoon cutting time upon sugar content of fresh cut alfalfa and the organic acid profile of ensiled alfalfa at two different locations in Michigan. The study was conducted over 2001-2002 at the Michigan State University farm in East Lansing (EL) and Upper Peninsula experimental station in Chatham (UP), MI. Alfalfa fields were divided into sections for morning (AM, between 0900 and 1030h) and late afternoon (PM, between1600 and 1700h) cuttings. Fresh samples were analyzed for sugar content and ensiled samples were analyzed for lactic and other organic acids. The experiment was arranged as a spilt-plot design with five replications. The sugar content of fresh samples was higher in the PM cuttings for both locations in both years. PM cut alfalfa silage resulted in increased lactic acid concentration compared to the AM cutting in only 3 out 12 cuttings for the two years at both locations. There was a significant correlation between sugar content and lactic acid concentration in the UP 2001 and EL 2002. Dry weather likely influenced both sugar content and lactic acid silage profile more than the time of cuttings during the day. Forage quality was not affected by cutting time of day.

DEDICATION

This research is dedicated to my parents for their years of patience and encouragement throughout my study abroad to achieve my goal. Special recognition to my wife, Nourah and my children, Saleh, Shahad and Abdulaziz for their patience and excellent support while I completed my program.

ACKNOWLEDGMENTS

I sincerely express my gratitude to my major advisor Dr. Richard. Leep as well as Dr. Suleiman Bughrara, co-advisor, for suggesting and supporting this project and for their excellent guidance throughout my Masters program at Michigan State University. My appreciation also goes to my graduate committee members including Dr. Herbert Bucholtz, Dr. Doo-Hong Min and Dr. Donald Penner for their interest in my research and valuable assistance in improving this project. A special thanks to Dr. Bucholtz, dairy cattle nutritionist in the Department of Animal Science who served as an external member, for his helpful advice to complete the silage data analyses. I want to expresses my appreciation to Timothy Dietz, research assistant in forages, for his great contribution through the field and lab work. I wish to thank Christian Kapp, research technician at the Upper Peninsula Experiment Station for his effort of collecting fresh and silage samples at the UP station. I also wish to acknowledge Tammy Webster at The Rumen Fermentation Profiling lab, West Virginia University, for performing the sugar analysis. Dr. Allen's lab, for their cooperation in providing the laboratory facilities to prepare silage samples. My appreciation to Dr. Rust's lab especially Bob Burnett, research technician for assistance analyzing alfalfa silage samples using HPLC. My gratitude is extending to Dr. Shasha Kravchenko for reviewing the statistical analyses. Finally, thanks to all CSS graduate students who helped me to achieve this project.

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LIST OF ABREVIATIONS

ADF	Acid Detergent Fiber
AM	Morning Cutting
Corr	Correlation
CP	Crude Protein
DM	Dry Matter
EL	East Lansing, location
Glm	General Linear Model
HPLC	High Performance Liquid Chromatography
MSC	Mean stage by count
MSW	Mean stage by weight
NDF	Neutral Detergent Fiber
PEAQ	Predictive Equation for Alfalfa Quality
PLH	Potato Leafhopper
PM	Afternoon Cutting
TNC	Total Nonstructural Carbohydrate
UP	Chatham, Upper Peninsula location

I. INTRODUCTION

Forage crops are grown primarily for feeding livestock, and can be harvested, stored or grazed directly by animals. Forage preservation, either as hay or silage, plays a critical role in assuring adequate nutritional value for livestock. In the United States, 25 million hectares of land are dedicated to silage and hay production and over 130 million metric tons (mt) of dry matter are produced (Albrecht and Hall, 1995). The term forage quality is defined as the capacity of forage to supply animal nutrient requirements. Buxton and Mertens (1995) defined forage quality in terms of performance of animals when fed herbage. It also includes the combination of chemical and biocharacteristics of forage nutrients and forage's potential to produce meat, milk, or wool.

Alfalfa (*Medicago sativa* L.), often called the "Queen of Forages" is a widely adopted crop across the world and has achieved this level of popularity because of its growth habit, reliability, winter survival, and rapid regrowth allowing multiple harvests each season. Alfalfa is the most important forage crop species grown in the United States and Canada. Annually, over 9 million hectares of alfalfa are cut for hay (1998 USDA Agriculture Statistics). Alfalfa is harvested either as dry hay or processed as silage. The most important characteristic of alfalfa is high nutritional quality values. It produces more protein per hectare than grain or oil seed crops and contains between 15 to 22% Crude Protein (CP) as well as a principal source of minerals and vitamins. These characteristics make alfalfa a desirable ration component for most farm animals (Barnes and Sheaffer, 1995). As part of a cropping rotation, alfalfa can increase subsequent crop productivity due to its ability to fix nitrogen through a symbiotic relationship with

Rhizobium meliloti (Vance et al. 1988). It may also improve soil water holding capacity and increase soil organic matter.

In Michigan, alfalfa is the primary source of forage (Borton et al. 1995) and is mainly used as silage on dairy farms. In 1999, alfalfa production in Michigan exceeded 3.6 million tons (Michigan Agriculture Statistics, 2000). Because of cold winters, alfalfa grown in Michigan commonly belongs to the dormant variety group of 3 and 4, which are considered winter hardy. The first harvest is usually in late May to early June and the number of harvests varies between three to four depending upon location within the state (Leep et al., 2002).

Improving alfalfa forage quality and persistence can be achieved by managing alfalfa carbohydrate content.

Carbohydrates

Photosynthesis is the process which plants captures light energy from sunlight to drive the conversion of carbon dioxide (CO_2), water, and minerals to oxygen and organic compounds. The initial product of photosynthesis is carbohydrate.

Carbohydrates are the primary energy source for ruminants and contribute 60-70% of the net energy used for milk production (Harris, 2002). Ruminants such as cattle and sheep have a complex digestive tract in which microbe's breakdown carbohydrates and produce volatile fatty acids, which are energy source for nutrients.

Carbohydrates can be classified either as structural or nonstructural. Structural carbohydrates are important in the formation of plant cell walls and characterized by their low digestion rates. Structural carbohydrates defined as neutral detergent fiber (NDF), which includes cellulose, hemicellulose, lignin, and portion of the pectin. Acid detergent

fiber (ADF) is another fiber value, which contains only cellulose and lignin. Total Nonstructural carbohydrates (TNC) consist of the cell contents, including sugars, starches and pectin and are considered easier to digest than cell wall components.

Alfalfa stores carbohydrates in the roots and crowns to be utilized after each cutting and to initiate regrowth after a dormancy period. Dormancy refers to a period of growth cessation as response to environmental factors (e.g. light, temperature). Fall dormancy helps to prepare the plant to survive the harsh winter. Fall dormancy occurs toward the end of the growing season (October) when cooler temperature prevails along with shorter day light period. New growth is initiated when more favorable growing conditions return in the spring (Mckenzie et al., 1988).

Plant sugar content is affected by environmental conditions such as: temperature, photoperiod and precipitation. Ueno and Smith (1970) investigated the influence of three temperature regimes (32/27 °C; 27/21 °C; and 21/15 °C day/night) in a growth chamber study on carbohydrate composition of three alfalfa cultivars grown for 35 d. They found TNC content higher at 27/21 °C than the other temperature regimes. In another growth chamber study, diurnal accumulation rate of TNC was higher when alfalfa was grown in short day length periods (10 h) compared to long ones (14 h) (Chatterton and Carlson, 1981).

Weather conditions prior to harvest can affect sugar content accumulation in alfalfa. If cloudy and wet conditions occur during the day before harvest, the initial carbohydrates and dry matter are likely to be lower (Curtis, 1944).

During early regrowth following harvest or dormancy period, the major source of carbon assimilates is carbohydrates stored in the crown and roots and during this time,

the shoots are the principal sinks. Starch most shows clearly seasonal fluctuations and is the main storage fraction. However, stress such as drought or cold, causes sucrose to become the major fraction. Nelson and Smith (1968) found sucrose to be the major fraction during early spring (April), late July and early August when the plants exhibited drought stress. Fall dormant alfalfa cultivars accumulate higher concentrations of carbohydrates than non-dormant cultivars (Castonguay et al., 1995). This accumulation is dependant upon the fall harvest timing. Hence, to ensure root reserve accumulation, proper fall harvest management needs to be considered.(Haagenson, 2000).

Alfalfa carbohydrates can be affected by insects. Potato Leafhopper (PLH), *Empoasca fabae* (Harris) is one of the most destructive insects attacking alfalfa in the eastern United States (Byers and Hower, 1976). PLH causes a reduction in photosynthesis, which eventually reduces the carbohydrate accumulation in the plant. PLH are capable of disrupting the normal flow of carbohydrates by their feeding behavior, which is caused by reducing the carbohydrate flow through the phloem (Lamp, 2003). Womack (1984) concluded that the rate of both transpiration and photosynthesis is reduced by disrupting the translocation of photoassimilates in the xylem and phloem. The symptoms of this damage in alfalfa include stunting, and leaf chlorosis (Manglitz and Ratcliffe, 1998). In addition, alfalfa seedling roots of plants exposed to PLH feeding have lower TNC (Shaw and Wilson, 1986). Thus controlling PLH is important in preventing the loss of alfalfa carbohydrates.

The relationship between sugar content and diurnal variation has been the subject of investigation by many researchers on several forage crops.

Diurnal variation in sugar content

During the normal photosynthesis process, plant sugar content peaks at the end of a sunny day. However, some sugars are lost during the night through respiration, leading to a diurnal variation of sugar content in the plant (Thomas et al., 2001).

The earliest studies of diurnal variation in carbohydrate content of crops were conducted on corn (*Zea mays* L.*)* and sorghum (*Sorghum bicolor* L.) (Miller,1924). He found that sugar content increased from 0400 to 0600 h and reached a peak between 1200 h and 1700 h, then started to decline again until the next morning. In addition, the sugar content of wheat (*Triticum aestivum* L.) seedling-leaves increased from 0900 h to 1600 (Krotkov, 1943). The time of harvest and nitrogen application effects on carbohydrate content of oats (*Avena sativa* L.) has been studied by Henry et al., (2000). They concluded that at each nitrogen level, the afternoon harvest contained higher carbohydrate content than the morning harvest.

Similar research was done in Oregon on pasture grasses where seventeen grass varieties were evaluated for sugar level (monosaccharides and disaccharides) over six cuttings in the 2001 growing season (Downing, 2002). The percentage of the sugar was higher in the PM than AM cutting time for all grass varieties.

Many researchers in different regions have studied alfalfa diurnal variation of sugar content. Curtis (1944) in New York reported a linear increase in carbohydrate content in alfalfa top growth from 4.3% to 6.1 % between AM and PM cutting. Research study conducted in Ames, IA, showed that the upper 7.8 cm of the plants contained the maximum concentration of reduced sugars between 1000 h and 1400 h (Allen et al., 1961). The water–soluble carbohydrate percentage in alfalfa ranged from

minimum at 0600 h to maximum level at 1200 h and finally decreased by 1800 h. (Holt and Hilst, 1969). However, some studies have found little difference between alfalfa AM and PM cutting times. Thomas et al., (2001) investigated sugar and starch content in AM and PM (second cutting) in the 2000 and 2001 growing seasons in western New York State. In 2000, the sugar content of the PM harvest was slightly higher than AM cutting (7.0% vs. 6.4%), and in the 2001 trial, the PM harvest contained significantly more sugar content (7.8% vs 6.3%). The differences between 2000 and 2001 may due the different weather condition.

Additional observations have shown that animals have a preference for PM cut alfalfa hay. Three ruminant species, sheep (*Ovis aries*), goats (*Capra hircus*) and cattle (*Bos taurus*) preferred alfalfa hay cut at sunset compared to hay cut at sunrise. The PM cut hay had a higher nutritive value, and consequently, animal production was increased by changing harvest management (Fisher et al., 2002)

The diurnal variation of the sugar content can influence silage fermentation. Thus, it so important to understand the process of making silage and how it can be impacted by sugar content.

Silage Fermentation Process

Silage is preserved forage which is stored in a silo under anaerobic conditions. The process of making silage includes several important steps starting in the field and culminating with the animal's consumption.

Silage fermentation is a microbial metabolic process, which requires anaerobic conditions, a substrate of soluble carbohydrate, appropriate moisture level and sufficient population of bacteria, which produces primary lactic along with acetic

acids (Rodriguez el al., 2000). Once the forage mass is packed properly, chemical changes occur as the environment shifts from an aerobic to an anaerobic phase.

The aerobic phase begins immediately after harvesting. At the beginning of the ensiling process, the pH is high (6.0 +) and plant cells can remain alive for a while, which allows aerobic bacteria to increase while the oxygen supply remains available. As a result, respiration breaks down plant sugars into carbon dioxide and water utilizing oxygen and releasing heat and CO_2.

The anaerobic phase, begins when the oxygen supply is depleted. The anaerobic bacteria population becomes active and increases in numbers in the oxygen free environment. The optimum temperature for silage bacteria is 37.7 °C (Bucholtz, 1999). The anaerobic bacteria ferment the sugars into lactic and other short chain volatile fatty acids. This increase in lactic acid reduces the silage pH to a range of 3 to 5. This low pH stops microbial activity and preserves the silage in a condition that is palatable to animals (Bolsen, 1995). The silage remains in a stable phase until there is exposure to oxygen during the feedout (Johnson and Harrison, 2001). During the process of fermentation, proteins are broken down into soluble non-protein nitrogen (Proteolysis). Proteolysis is dependant upon pH, temperature, moisture level and forage species. (Silage process steps are presented in Appendix Table A.1).

High quality silage requires controlling the factors essential for complete silage fermentation. The optimum moisture level of alfalfa at ensiling ranges from 50-70%. Excessive wet silage (> 70% moisture) encourages the growth of undesirable Clostridial bacteria which convert plant sugar and/or plant protein to butyric acid and amines causing dry matter loses and a higher pH (Mathews, 1999), resulted lowers

silage quality and makes the silage undesirable for animal consumption. In contrast, excessive dry forage (< 50 % moisture) may prevent sufficient growth of desirable silage bacteria causing a reduction in silage acids (Bucholtz, 1999). Optimum pH levels and elimination of oxygen are important factors in preventing the growth of yeast and molds. In general, higher concentrations of lactic and acetic acids resulted in more stable silage (Bolsen, 1995).

Silage may be made from any crop, which can be used as green forage or hay; however, silage crops should be selected for their agronomic and animal nutritional characteristics. Alfalfa silage is high in crude protein, calcium and phosphorus but it has limited sugar available for fermentation.

The importance of the sugar on alfalfa silage quality

Silage quality is determined in part by the amount of sugars available for fermentation (Church, 1991). Lactic acid bacteria ferment sugars and produce lactic and acetic acids, which reduce pH and keep the silage in good condition (Bucholtz, 1999). In general, forages with low concentrations of soluble carbohydrates may require an additional source of carbohydrates such as ground grain to enhance its fermentation (Harris, 2002). Sugars, also enhance the nutritional value and palatability of the silage and are a source of energy required for animal nutrition.

Alfalfa cutting time can be managed to ensure high sugar content for better silage fermentation. For example, alfalfa silage made from late afternoon cutting may result in better fermentation due to the higher sugar content. Melvin (1965) found that PM cutting alfalfa was significantly higher ($P<0.01$) in sugars and starch, resulting in higher lactic acid and lower silage pH.

Harvesting for Silage

Harvesting at the right time is the first step in making silage. Alfalfa should be harvested when the crop growth stage is between bud and early flower. At this stage of development, the crop has reached the optimum content of neutral detergent fiber (NDF). In general, it is recommended to chop alfalfa for silage when DM ranges between 30% and 35%, NDF between 36% and 42%, and CP between 20% and 25% (Johnson and Harrison, 2001).

Predicting the time of harvest is important to ensure alfalfa quality. One method used to predict cutting time is growing degree days [(max temp + min temp)/2-base temp]. Another method is called Predictive Equation for Alfalfa Quality (PEAQ), which is based upon alfalfa maturity, stage and plant height. Researchers often use Mean stage by count (MSC) or Mean stage by weight (MSW), described by Kalu and Fick (1981) based on the number or weight of stems in different maturity stages. Each stage may be defined according to a scale, which classifies from 0 to 9.

The recommended particle length of forage crops chopped for silage varies among species; alfalfa should be chopped at a length of 2.54-5.08 cm. However, for corn silage, 15% to 20% of the particles should be greater than 3.81 cm (Kung and Neylon, 2001).

Objectives

The objectives of this study are to:

1. Determine the extent to which sugar content in alfalfa is influenced by AM versus PM cutting.
2. Determine the effect of AM versus PM cutting time on lactic acid concentration of ensiled alfalfa.
3. Study the effect of latitude on diurnal variation of sugar content by comparing the AM versus PM cutting times at East Lansing (42 degrees latitude) and Chatham (46 degrees latitude).
4. Determine the effect of AM versus PM cutting on forage quality, which include acid detergent fiber, neutral detergent fiber, and crude protein.

II. MATERIALS AND METHODS

Site Description

This study was conducted on established alfalfa fields during the 2001 and 2002 growing seasons at: (i) The Michigan State University Farm in East Lansing (EL), Ingham County, MI (42°, 47′ N, 84° 36' W, elevation of 258 m) on a Capac loam soil (fine-loamy, mixed, mesic Aeric Ochra-qualf) and (ii) The Michigan State University Agricultural Experiment Station -Upper Peninsula (UP) in Chatham, Alger county, MI 595 km (370 miles) north of East Lansing (46° 33′ N, 86° 55' W, elevation of 267 m) on a Stony loam soil (Typic Haplorthod). Soil test results at the EL site were 41 kg/ha of P and 132 kg/ha of K and soil pH 5.7 while results at the UP site were 21 kg/ha of P and 82 kg/ha of K and soil pH 7.2. The UP site has higher rainfall accumulation than EL, and is cooler especially in the spring and late summer than EL site.

Harvesting and Sampling

The EL site was an alfalfa field of cultivar Pioneer 5312 and the UP site was a field of cultivar Mycogen Multiplier. Both cultivars are fall dormancy 3. At each site, a 400 m^2 strip was selected from the alfalfa field for the cutting treatments. Each strip was divided into two sections (cutting times). (i) Morning cutting (AM), between 0900 and 1030 h (immediately after the dew dried) and (ii) Afternoon cutting (PM) between 1600 and 1700 h (Eastern Time). At each cutting time, samples were collected immediately after cutting for analysis as fresh hay. Samples were also collected for silage after alfalfa moisture was dried to approximately 60%.

There were three harvest events per season (2001-2002) at each site. Since the harvest dates were dictated by weather conditions and crop stage of development, the interval between first, second, and third harvests varied. The stage of development at harvest ranged from late bud to early flower.

Collection of fresh Samples

Fresh samples were collected from the freshly cut alfalfa windrow immediately after each cutting time. Five freshly mowed samples (500 g of each) were randomly collected in paper bags and frozen in a freezer (-15 °C) to limit sugar loss from respiration. Fresh alfalfa samples were analyzed for sugar content, NDF, ADF, and CP.

Preparation of silage samples

Five mini-silos (30-45 cm ht by 10.16 cm diameter PVC pipe) were packed with alfalfa collected from each of the AM and PM cutting times at 63-65% moisture level. Packing was done by placing the chopped alfalfa into one end of the mini-silo and rapidly pressing all the air out of the mini-silos as they were filled (Fig 1). The mini-silos were then sealed and kept at room temperature (21- 26 °C) for more than 60 d to ensure complete fermentation (Burns, 2001). Alfalfa silage samples were analyzed for lactic and other organic acids.

2001

The EL field was cut using a mower conditioner on 13 June, 16 July and 21 August and the UP field was cut on 20 June, 30 July and 10 September. The silage samples were prepared the day following cutting.

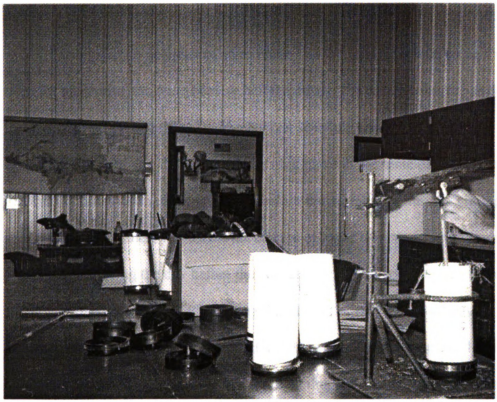


Fig 1. Packing the PVC mini-silos.

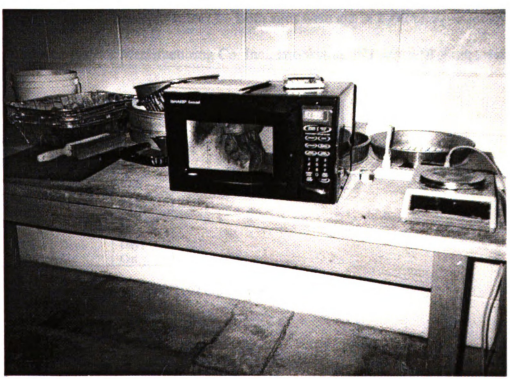


Fig 2. Microwave oven and scale to determine the moisture level.

2002

Some changes in protocol were made in 2002, which include using a microwave oven for measuring moisture content before packing the ensiled samples as described by Brusewitz et al., (1993) (Fig. 2). The harvest schedule was also altered from the first year. In 2001, AM and PM cutting times took place in the same day where in 2002, the PM cutting time occurred the afternoon before the morning of the AM cutting time. This method more accurately represents the change of sugar content due environmental conditions occurring the day before the AM cutting. The harvest design included two strips with one specified for PM cutting (day 1) and other for the AM cutting on the following day (day 2). The area around the strips was harvested on day 1 to allow maximum air circulation (Fig.3). The 2002 cutting dates in the EL field were on 19/20 June, 17/18 July, and 21/22 August, and in the UP field were on 2/3 July, 5/ 6 August and 30 September/1 October (Table1). Both sites were cut in 2002 using a Carter Flail Harvester (Carter Manufacturing Co. Inc., Brookston, IN) with a 91.4 cm swath.

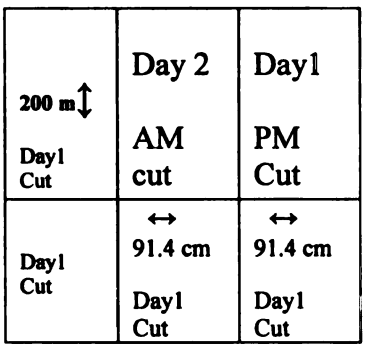


Fig.3 The harvest design of the 2002 experiment.

Alfalfa Analyses

Fresh samples

Fresh samples were analyzed for sugar content and nutritive content including ADF, NDF and CP. The frozen samples were freeze-dried in a Tri-Philizer MP (FTS, Kinetics, Stone Ridge, NW) freeze drier for 4 d to a moisture level below 10%. Dried samples were then ground to pass through a 2 mm screen using a Wiley Grinding Mill (Authur H. Thomas Co. Philadelphia, PA) and then passed through a 1mm screen using a UDY Cyclone Mill (Udy Mill Corp., Fort Collins, CO).

Sugar analysis was completed by the Rumen Fermentation Profiling Lab, West Virginia University based on the method of partitioning of neutral detergent soluble carbohydrates using 80:20 (v/v) ethanol/water as described by Hall et al., (1999).

Total nitrogen was determined for the subset by the Hach modified Kjeldahl procedure (Watkins et al., 1987). Hach procedure based on digesting the sample using sulfuric acid/ hydrogen peroxide to reduce all the nitrogen to ammonia without salt or metallic catalysts used in Kjeldahl method. CP was estimated by multiplying total N by 6.25. The Goering and Van Soest (1970) method was used for NDF and ADF determination with the addition of 1 ml of alpha-amylase to the neutral detergent solution for the breakdown of starch.

Silage samples

Since lactic and acetic acids are the major fermentation products, alfalfa silage samples were analyzed for the lactic and acetic acids. However, proprionic and butyric acids, and silage pH were also determined to evaluate the quality of the silage fermentation. This analysis was conducted using HPLC (High Performance Liquid

Chromatography) (Waters Chromatography Division, Milford, MA). This method is based on the general procedure of Canale et al., (1984) modified by Rodrìguez- Carìas (1995).

Silage analysis was performed following completion of fermentation by discarding the upper 5 cm of spoiled plant material and obtaining a 500 sample from the center portion of the mini-silo. A 50 g sub sample of silage was placed into a polyethylene bag with 450 ml distilled water. The sample was then hydrolyzed for 5 min in a Tekmar blender (3500 Stomacher, Tekmar, Cincinnati, OH). The sample was then filtered through four layers cloths to obtain an extract. A portion of the extract was used to determine pH and the other portion was placed in 4 ml sample vials and centrifuged at ($26,000 \times g$) for 30 min. The supernatant liquid was transferred into HPLC sample vials for the acids analysis. Graphic peaks were obtained for each acid in the sample. Dry matter content of each sample was determined by drying 5 g of sample in aluminum pans at 105°C for 12 hr.

Statistical Analysis

The experimental design was a spilt-plot with cutting time (AM or PM) as a whole plot factor with 5 replications and harvest number (1^{st}, 2^{nd} or 3^{rd} harvest) as a subplot factor. Data obtained from sugar and silage fermentation analyses were used to test the statistical significance of the treatments effects (cutting time). Analysis of variance (ANOVA) was obtained using proc Glm. The treatments and the harvest number were considered as a fixed effect. The normality of the data was checked using Proc Univariate.

The statistical model is:

$\mathbf{Y_{ijk}=\mu+ Trt_i+ Rep_j+ + Er_1+ Cut_K+ (Trt*Cut)_{ik}+Er_2}$, Where:

Y_{ijk}= Dependent variable.

μ= General mean.

Trt_i= Treatment effect (i=1-2) (AM/ PM).

Rep_j= Replication effect (j=1-5).

Er_1= $(Trt*Rep)_{ij}$ = The interaction between treatment and replication used as the. whole plots error to test significance of treatment effect.

Cut_k= harvest number effect (k=1^{st}, 2^{nd}, 3^{rd}).

$(Trt*Cut)_{ik}$= The interaction between treatment and harvest dates.

Er_2= The subplot error.

The sugar analysis data for both sites were combined and the location effect was tested. Treatment means were compared using Tukey procedure. The correlation between sugar and lactic acid concentration in each location was obtained using Proc Corr. All the statistical computations were performed using SAS (SAS, 2000).

III. RESULTS AND DISCUSSION

Weather Records

a) East Lansing (EL)

Environmental stresses such as high temperature or lack of rainfall may have reduced sugar accumulation in the alfalfa plant. The EL weather records for 2001, 2002 and the 30-year average show the precipitation patterns deviated from the 30-year average throughout the season and between years. Precipitation levels in July and September were different in 2001 and 2002 (Fig.4). In 2001, the interval between harvestings was 34 to 35 d and the total precipitation in the interval between the first to the second and the second to the third harvest was 6.5 cm, which was below the 30- year average. The monthly maximum and minimum air temperatures were near to the 30-year average. However, for individual cutting days, maximum air temperatures were above normal for the first and second cuts. In 2002, the interval between harvestings was 28 to 35 d. The total precipitation in the intervals between the first to the second, and the second to third harvest were 3.5 and 8.48 cm, respectively (Table 1). The maximum air temperatures for individual cutting days were similar to the 30 -year average.

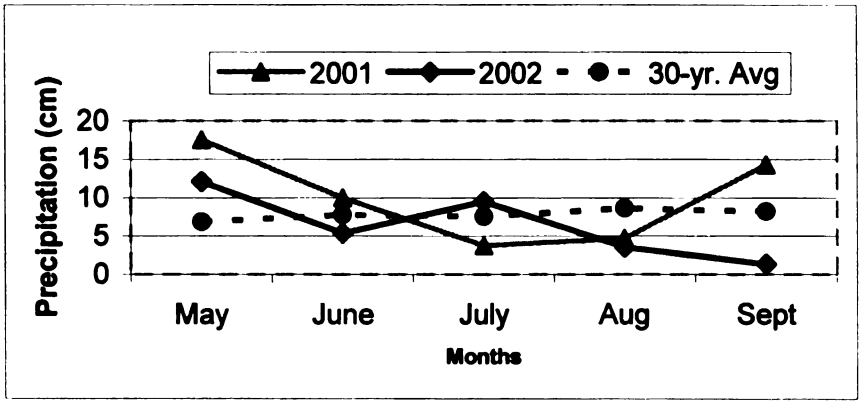


Fig 4. Monthly precipitation in 2001-2002 compared to the 30- year average for the EL site.

b) Chatham (UP)

The precipitation patterns at Chatham (UP) were different in 2001 and 2002 (Fig.5). In general, monthly precipitation levels were similar to the 30-year average; however, for individual cutting dates, in 2001the interval between cutting dates ranged from 40 d and 42. In 2002, the interval between the second and third harvest was longer than usual and the precipitation in this interval was 50% less than the first interval (12.6 vs 23.2 cm). The final cut in 2002 was delayed due to the lack of moisture between the second and the third harvests. The maximum temperature in May was about 6 degrees lower and the minimum was 3 degrees lower than the 30-year average (Table 1).

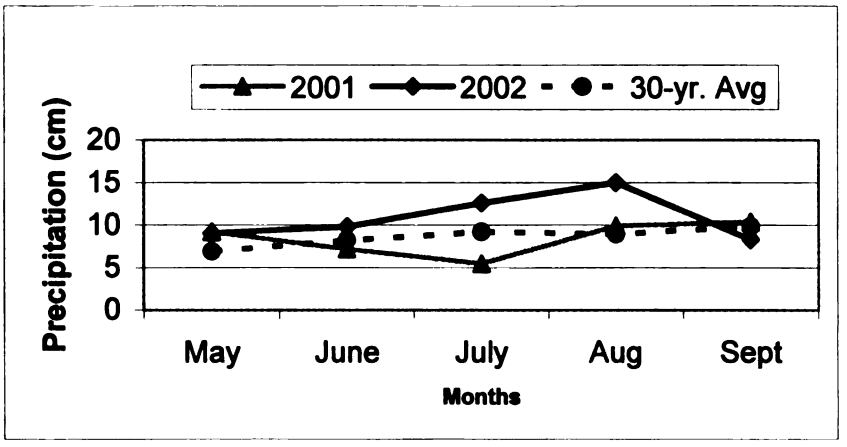


Fig 5. Monthly precipitation in 2001-2002 compared to the 30- year average for the UP site.

Table 1. Harvest dates, intervals between harvest dates (d), precipitation in each interval (cm), temperature (°C) and weather conditions for each harvest day (s) at EL and UP sites over 2001-2002.

Location	Harvest No.	Harvest date(s)	The interval (d) between Cutting days	The percp. in each Interval (cm)	Temp.(°C) In each cutting day Max - Min	Weather conditions in each harvest day
		(2001)				
EL	1	13 June	n/a ‡	n/a	28.3 -16. 6	Partly cloud at pm
	2	16 July	34	34	30.5 -13.9	Partly cloud at pm
	3	21 August	35	35	25.5- 10.0	Sunny
UP	1	20 June	n/a	n/a	23.3 - 6.6	Sunny
	2	30 July	40	40	22.8 - 8.9	Sunny
	3	10 Sep.	42	42	18.4 - 6.5	Sunny
		(2002)				
EL	1	19/20 June	n/a	n/a	29.6 -13.5 / 32.1- 16.1	Sunny
	2	17/18/July	28	28	30.4 -17.8 / 29.5 -18.1	Sunny
	3	21/22/Augst	35	35	27.5-11.2 / 25.4 -19.1	Partly cloud &humid
UP	1	2/3 July	n/a	n/a	35.5 - 21.1 / 28.9-16.1	Sunny at pm
	2	5/6 August	34	34	25.5 -7.0/ 20 -6.1	cloudy at pm, sunny at am
	3	30 Sep/Oct.st	56	56	17.7 -6.6 / 22.7-12.7	cloudy, very humid at am

‡= *Data are not applicable*

Table 2. Analysis of Variance and *p-Value* of F-Test for Morning (AM) vs. afternoon (PM) cutting treatments, replications, and number of cut at EL and UP during the 2001-2002 growing season for sugar content (%) and lactic acid concentration (%).

Sources of Variation	(2001) EL					UP			
	Sugars			Lactic acid		Sugars		Lactic acid	
	DF	MS	*P-value*	MS	*P-value*	MS	*P-value*	MS	*P-value*
Rep.	4	0.36	------	0.31	-------	0.28	------	1.43	-------
Trt.(am/pm)	1	7.10	0.004	1.35	0.050	4.74	<0.001	4.32	0.087
Error (1)	4	0.20	-------	0.17	------	0.05	-------	0.85	-------
Cut	2	24.28	<0.001	6.25	<0.001	0.22	0.499	0.17	0.795
Cut*Trt	2	0.10	0.527	0.10	0.707	0.26	0.452	1.74	0.137
Error (2)	16	0.14	------	0.30	------	0.31	-------	0.74	-------
	(2002) EL					UP			
	Sugars			Lactic acid		Sugar		Lactic acid	
	DF	MS	*P-value*	MS	*P-value*	MS	*P-value*	MS	*P-value*
Rep.	4	0.27	-------	2.40	--------	0.068	--------	1.42	------
Trt.(am/pm)	1	11.6	<0.001	0.36	0.662	1.36	0.008	1.09	0.290
Error (1)	4	0.043	-------	1.65	-------	0.058	------	0.73	------
Cut	2	3.24	<0.001	16.08	<0.001	10.78	<0.001	38.77	<0.001
Cut*Trt	2	0.67	0.007	5.74	0.022	2.09	<0.001	23.59	<0.001
Error (2)	16	0.097	--------	1.18	-------	0.06	-------	1.07	-------

2001-Laboratory Results

Sugar Analyses

The analysis of variance (ANOVA) in table 2 shows alfalfa sugar content at (EL) and (UP) sites was significantly higher ($p<0.01$) in the PM cutting compared to the AM cuttings. There was no interaction between the harvest number and the AM and PM cutting times (Table 2).

Comparing sugar content of AM and PM cutting times, the PM cutting was significantly higher only in the second and third harvest in EL ($p<0.01$), and the PM cut was higher only in the first harvest ($p<0.05$) in the UP site. In general, the sugar content at the UP site did not vary between harvests (Fig.6). However, the sugar content in the EL third harvest was lower than the first and second harvests. The lower sugar content in the third harvest at EL may be due to the drought stress during July (Fig 4). Plants close their stomata in dry conditions to conserve moisture, but they also eliminate the supply of carbon dioxide necessary to synthesize carbohydrates during photosynthesis (Hopkins 1999). Combining data from both sites shows alfalfa sugar content to be numerically higher in EL compared to the UP, but not statistically significant.

First year results showed a higher numerical sugar content in the PM cutting of fresh alfalfa. The PM cut was significantly higher in sugar content for only 50% of the total cuttings at both sites (Fig.6). These results concur with several studies conducted in other states in the US such as Allen et al., (1961) in Iowa, and the second year data from Thomas et al., (2001) in New York as well as other studies done in different countries such as Melvin (1965) in Melbourne, Australia.

2001

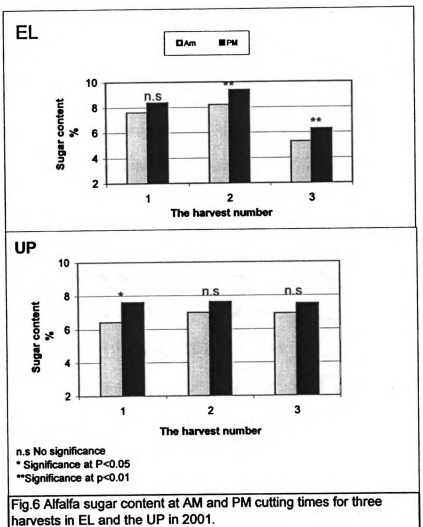


Fig.6 Alfalfa sugar content at AM and PM cutting times for three harvests in EL and the UP in 2001.

Silage Fermentation Analyses

The lactic acid concentration of alfalfa silage in the PM cut was significantly higher than the AM cut at both sites ($P<0.5$ in EL; and $P<0.10$ in UP). There was no interaction between the harvest number and the AM and PM silage treatments (Table 2). There was a negative correlation between sugar content and lactic acid concentration in the EL site. However, there was significant correlation ($P<0.01$) between sugar content and lactic acid concentration at the UP site and the correlation coefficient is 0.54083.

Comparing the lactic acid concentration and the harvest number, the lactic acid in the PM cutting was significantly higher than the AM cutting only in the second harvest at EL ($p<0.05$) and in the first harvest at the UP site ($P<0.01$) (Fig.7). There was a slight decline in lactic acid for the PM cutting in the UP third cutting, but not significant. The lack of plant moisture can affect silage fermentation by preventing the silage pH from the falling to levels that allow for optimal anaerobic microbial activity (Mathews 1999).

The data from EL 2001 in table 3 shows that in the first and second harvests, silage samples may have been too dry (55% and 62% DM respectively), which may explain the drop in lactic acid in these harvests. Another argument which may explain the lower lactic acid concentration could be the result of improper handling of the materials. For example, alfalfa harvested in 2001 was not chopped before ensiling. Rodriguez et al., (2000) indicated that the number of lactic acid bacteria increases in silage made from chopped alfalfa compared to silage made from whole alfalfa. Since the harvested materials were ensiled as a whole alfalfa in EL in 2001, this may have resulted in a situation where a rapid increase of lactic acid bacteria population did not occur with

subsequent lower production of lactic acid. Thus, the negative correlation in EL was most likely due to lack of lactic acid produced in the alfalfa silage at both cutting times.

In spite of low lactic acid concentration in the 2001 silage samples, silage pH was in the appropriate range (3 to 5). Silage pH was not statistically different between AM and PM harvest time. The butyric acid concentrations in silage samples from both sites were low, which indicate good fermentation occurred (Table 3).

Forage Quality Analyses of fresh samples

There were no significant differences between AM and PM cutting times for Neutral Detergent Fiber, Acid Detergent Fiber and Crude Protein (Table 4). Since there were no significant differences in the forage quality analyses in 2001, this analyses were performed only in 2001.

2001

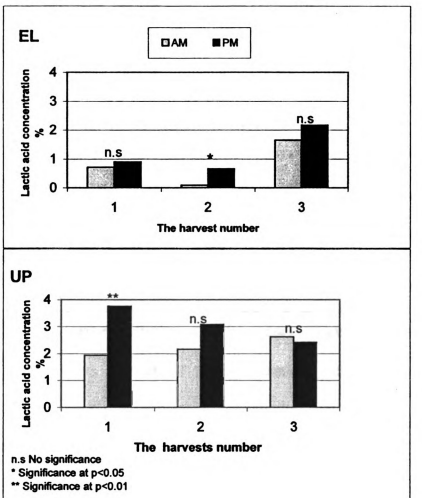


Fig.7 Lactic acid concentration in alfalfa silage made from AM and PM cutting times for three harvests in EL and the UP in 2001.

Table 3. Mean values for fermentation analysis and sugar levels for AM vs.PM alfalfa harvested in EL and UP in 2001 growing season.

Location	Harvest No.	Cutting time	Fresh-sample sugar (%)	Fermentation analyses of ensiled alfalfa					
				DM (%)	Lactic Acid (%)	Acetic Acid (%)	Prop. Acid (%)	Butyric Acid (%)	pH
EL	1	AM	7.61 a	54.16	0.71 a	0.20 a	0.07 a	0.00 a	5.05 a
		PM	8.37 a	45.53	0.90 a	0.16 a	0.10 a	0.04 a	5.14 a
	2	AM	8.20 a **	61.86	0.10 b *	0.07 a	0.06 a	0.0 a	5.37 a
		PM	9.36 b	34.51	0.66 a	0.12 a	0.10 a	0.04 a	5.33 a
	3	AM	5.28 b **	37.72	1.65 a	0.40 a	0.21 b**	0.00 a **	4.95 a
		PM	6.27 a	30.6	2.17 a	1.0 a	0.10 a	0.13 b	5.09 a
UP	1	AM	6.42 b *	27.1	1.93 b **	3.64 a	0.35 a	0.59 a	4.91 a
		PM	7.59 a	29.18	3.76 a	2.36 a	0.22 a	0.03 a	4.73 a
	2	AM	6.98 a	46.60	2.16 a	0.64 a	0.08 a	0.00 a	4.48 a
		PM	7.62 a	35.75	3.08 a	0.63 a	0.10 a	0.00 a	4.42 a
	3	AM	6.93 a	34.47	2.62 a	1.31 a	0.13 a	0.01 a	4.86 a
		PM	7.51 a	33.37	2.42 a	0.71 a	0.09 a	0.00 a	4.77 a

Mean values within columns for each location, for AM and PM cutting time, and for each harvest number followed by different letters are significantly different.
Tukey * *: Significant at P<0.05*
** *: Significant at P<0.01*

Table 4. Crude protein (CP), Acid detergent fiber (ADF), and Neutral detergent fiber (NDF), for AM vs.PM alfalfa harvested in EL 2001.

Harvest No.	Cutting Time	Alfalfa Forage Quality analyses (%)		
		ADF	NDF	CP
1	AM	40.5 a‡	513 a	19.1 a
	PM	39.1 a	50.2 a	18.4 a
2	AM	32.9 a	43.2 a	19.9 a
	PM	30.9 a	41.0 a	20.3 a
3	AM	28.4 a	39.1 a	24.0 a
	PM	26.7 a	36.2 a	24.0 a

‡ *Mean values within columns follow by the same letters are not significantly different (TukeyP < 0.05).*

2002-Laboratory Results

Sugar Analyses

Sugar content in the fresh alfalfa samples was significantly higher ($p<0.01$) in the PM cuttings compared the AM cuttings for both sites. The analysis also shows there was a significant interaction between cutting time treatments and harvest number. Comparing the treatment means, EL results showed PM cut alfalfa to be significantly higher in sugar content in the first and third harvests ($P<0.01$) and second harvest ($P<0.05$). The PM cutting in the UP was significant higher only in the third harvest ($P<0.01$). However, the AM second cutting was significant higher in sugar content compared to the PM cut (Fig 8). This increase in the level of sugar in the AM cutting may be related to the cloudy overcast weather condition on the day prior to the PM cut (Table 1). Curtis (1944) concluded that initial carbohydrates and dry matter are likely to be low if cloudy and wet conditions occur during the previous day before cutting. Combining the results from both sites, alfalfa sugar content in the PM cutting was significantly higher ($P<0.01$) than the AM cut.

The second year results show that alfalfa sugar content was higher in all PM cuttings at EL but only significantly higher in the third cutting at the UP site. The high sugar content in the AM second cutting in the UP was most likely due to cloudy weather condition the day before the PM cuttings (Table 1).

2002

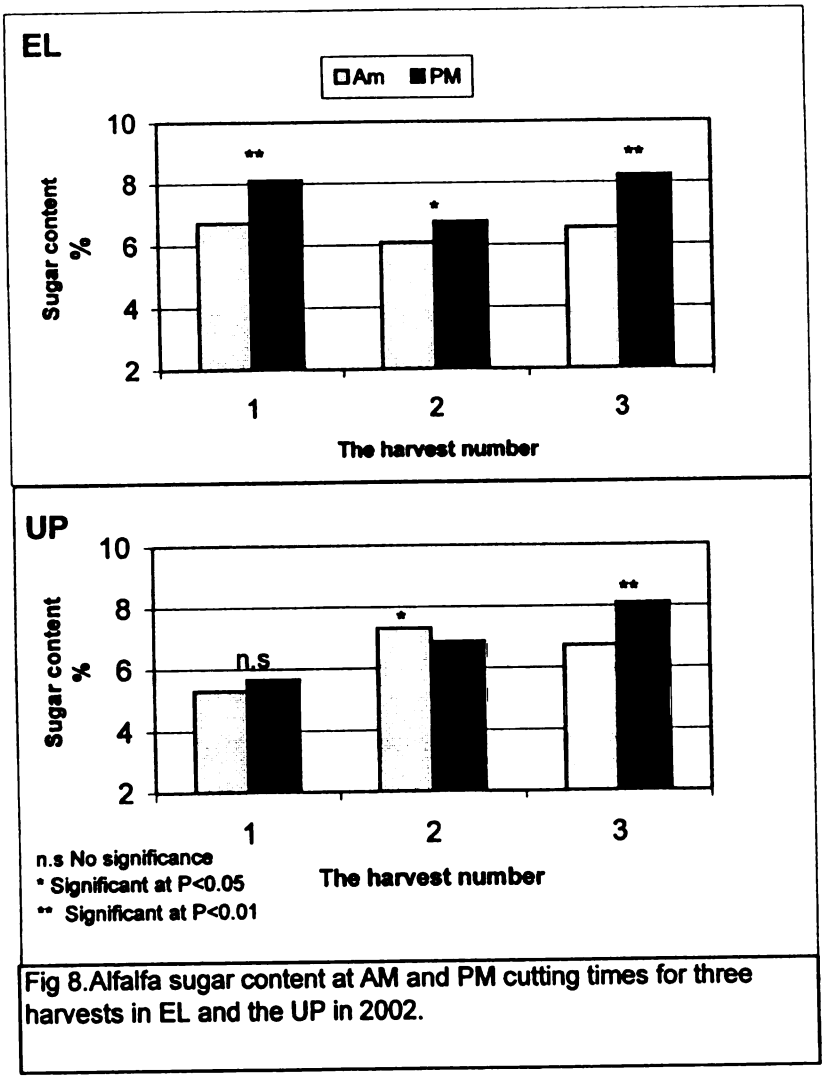


Fig 8.Alfalfa sugar content at AM and PM cutting times for three harvests in EL and the UP in 2002.

Silage Fermentation Analyses

Even though there was significantly greater sugar content in the PM fresh cut at EL, there was no significant difference in lactic acid concentration between AM and PM cutting times. In EL, there was a significant correlation between sugar content and lactic acid concentration ($P<0.05$) and the correlation coefficient is 0.36119. However, there was no correlation between sugar content and lactic acid concentration at the UP.

Data analysis for the lactic acid concentration at the UP shows that there was a significantly higher level of lactic acid in the third harvest (Fig 9). Results from New York study by Thomas et al., (2001), agree with our second year results that there was no relationship between AM and PM cuttings in lactic acid concentration. There was a significant interaction between AM and PM cutting treatments and harvest number for lactic acid concentration (Table 2). However, unlike other experiments conducted in other locations, the UP morning cut was significantly higher in lactic acid concentration ($P<0.01$) than the afternoon cut in the first harvest.

In addition, there were no significance differences in silage pH at EL in the first and second harvests while the third harvest was significantly different ($P<0.01$). There was a significant difference ($P<0.01$) in silage pH in the first and third harvest of the PM cutting at UP site (Table 5).

As indicated earlier, silage quality decreases with high moisture level (Bucholtz 1999). Thus, the excessive moisture content (80%) of alfalfa silage in third harvest at the UP site resulted in low lactic acid concentration because these conditions promoted the production of butyric acid causing an elevation of the silage pH. Acetic acid concentration was significantly different ($P<0.01$) at both sites.

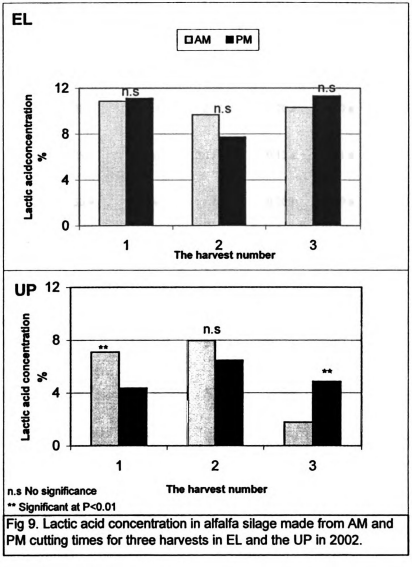
Lactic acid concentration in alfalfa silage harvested in 2002 was much higher than in 2001. For example, the differences between 2001 and 2002 lactic acid concentration in the AM cutting time at EL first harvest was 0.71% and 10.83% respectively and PM cutting time at the same harvest was 0.90% and 11.10 respectively (Tables 3 and 5). These results may be due to the moisture level inside the mini-silos in 2001 samples. The excessive dry forage in the first and second cuts in 2001in the EL alfalfa silage was a factor causing the lower lactic acid. On the other hand, alfalfa harvested in 2001was not chopped before it was ensiled. Hence, long stem ensiled alfalfa may have resulted in lower lactic acid concentration. Additionally, the temperature of the ensiled sample might have impacted the lactic acid bacteria function.

These results show there may be an advantage for Michigan farmers to harvest alfalfa in the afternoon instead of the morning to obtain higher sugar levels. However, from a practical standpoint there are several limitations that should be considered. First, Michigan has relatively high rain fall during the growing season compared with many of the western states. Therefore, harvesting at a particular time of day may be impractical, depending on the current weather conditions. Second, cool weather at the first harvest may limit the growth of desirable bacteria after harvesting resulting in poor silage fermentation, negating any potential gains intended for ensiling. Additionally, there is currently no value placed on higher sugar level of hay in the market place.

Silage fermentation is a complex process that is influenced by many different factors. A topic for future studies should focus on more of the factors which affect fermentation. For example, the sugar content of the silage should be analyzed to determine how much sugar remains after fermentation. Additionally, silage may be

analyzed for the total number of the lactic acid bacteria in each sample to determine if the poor fermentation is due to insufficient bacteria population. In addition, close attention needs to be placed on ensiling alfalfa at optimal moisture content.

2002



n.s No significance

** Significant at P<0.01

Fig 9. Lactic acid concentration in alfalfa silage made from AM and PM cutting times for three harvests in EL and the UP in 2002.

Table 5. Mean values for fermentation analysis and sugar levels for AM vs.PM alfalfa harvested in EL and UP in 2002 growing season.

Location	Harvest No	Cutting Time	Fresh-sample Sugar (%)	Fermentation analyses of ensiled alfalfa					
				DM (%)	Lactic Acid (%)	Acetic Acid (%)	Prop. Acid (%)	Butyric Acid (%)	pH
EL	1	AM	6.72 b **	28.1	10.83 a	2.30 a	0.21 a	0.00 a	4.28 a
		PM	8.10 a	28.1	11.10 a	2.12 a	0.20 a	0.00 a	4.20 a
	2	AM	6.07 b *	28.1	9.65 a	2.17 a **	0.13 a	0.01 a	4.04 a
		PM	6.74 a	30.9	7.73 a	1.64 b	0.20 a	0.00 a	4.01 a
	3	AM	6.54 b **	25.3	10.30 a	2.65 a **	0.34 a	0.00 a	4.22 a**
		PM	8.22 a	28.3	11.30 a	1.90 b	0.27 a	0.00 a	3.95 b
UP	1	AM	5.29 a	28.4	7.08 a **	2.44 a	0.27 a	0.00 a**	4.37 a**
		PM	5.65 a	29.7	4.34 b	1.65 a	0.28 a	1.77 b	5.01 b
	2	AM	7.30 a *	26.7	7.96 a	1.42 a	0.22 a	0.00 a	4.30 a
		PM	6.85 b	28.1	6.46 a	1.27 a	0.21 a	0.00 a	4.32 a
	3	AM	6.72 b **	21.4	1.76 b **	3.60 a **	0.42 a	1.54 a	5.58 a**
		PM	8.10 a	20.5	4.85 a	2.28 b	0.56 a	0.80 a	5.12 b

Mean values within columns for each location, for AM and PM cutting time, and for each harvest number followed by different letters are significantly different.

*Tukey * : Significant at P<0.05*

*** : Significant at P<0.01*

IV. SUMMARY

Alfalfa cut in the afternoon had higher sugar content than alfalfa cut in the morning in 7 of 12 harvests at two sites over two years. In addition, silage made from afternoon harvested alfalfa in 2001 resulted in better fermentation than silage made from alfalfa harvested in the morning under Michigan growing conditions. However, the 2002 data did not provide any evidence for an advantage of cutting in the late afternoon for increasing silage lactic acid concentration.

Weather conditions such as drought stress and in some cases, cloudy conditions, may have reduced the sugar content of harvested alfalfa. Dry ensiled materials were also a factor in this study during 2001, which may have caused lower lactic acid concentration. We also found that high moisture content in alfalfa silage lowered the concentration of lactic acid. Comparing sugar levels of the two sites showed there was a latitude effect on sugar content in alfalfa cut in the AM and PM time only in 2002.

Lastly, this study did not present any consistent evidence for an advantage for cutting at any particular time of day on alfalfa forage quality factors including CP, NDF, and ADF.

V. APPENDICES

Table A.1. Summary of silage process in aerobic and anaerobic phases.

Aerobic Phase	Anaerobic Phase
Harvest. ↓ Availability of Oxygen. ↓ Respiration phase. ↓ Aerobic bacteria activity. ↓ Loss of nutrient. ↓ Release carbon dioxide, heat, and water. ↓ Proteases.	Filling and packing. ↓ Free of Oxygen + Optimum moisture. ↓ Anaerobic bacteria activity. ↓ Fermentation Phase. ↓ Produce Lactic acid. ↓ Low pH. ↓ Stop microbial activity. ↓ Stable and palatable silage.

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