Effect of Rain Leaching on Chemical Composition of Alfalfa Hay

Mercedes M. Garcia de Hernandez
Utah State University

EFFECT OF RAIN LEACHING ON CHEMICAL COMPOSITION OF ALFALFA HAY

by

Mercedes M. García de Hernández

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I dedicate this work to my parents to whom I am very thankful for the love of nature and the spirit of diligence which were effectively given to me throughout my life. My final dedication is for those people involved in this interesting and fascinating field, Animal Nutrition.

Mercedes M. García de Hernández

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ABSTRACT

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Composition of Alfalfa Hay

by

Mercedes M. Garcia, Master of Science

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Major Professor: Paul V. Fonnesbeck
Department: Animal, Dairy and Veterinary Sciences

Yield and chemical changes of second-cutting alfalfa hay treated with artificial rain were determined in a 2 x 3 x 2 factorial experiment. Factors were 2 stages of maturity (1 = late vegetative; 2 = early bloom), 3 levels of artificial rain applied (1 = no rain; 2 = low or approximately 5 mm; 3 = high or approximately 20 mm), and 2 times of applying artificial rain (1 = when drying forage was 40-60% dry matter; 2 = when drying forage was 60-75% dry matter). Thirty samples of alfalfa were collected at the 2 stages of maturity when the alfalfa was fresh cut, pre-sprinkled, pre-baled and pre-feeding. Alfalfa samples were analyzed for dry matter, nitrogen, ash, plant cell walls, cellulose, hemicellulose, lignin, total lipids and acid insoluble ash. Available carbohydrates and soluble ash were calculated.

Yield of dry matter increased with advancing maturity. Plant cell content fraction was lowered but plant cell wall constituents were increased with advancing stage of growth.

Artificial rain significantly affected chemical composition of alfalfa hay. Available carbohydrates, soluble ash and ash decreased due to the effect of leaching. Total lipids was slightly reduced by leaching, while protein content was not changed. Cellulose, lignin and cell wall fractions of alfalfa hay increased consistently under the effect of artificial rain. Sprinkling and processing time did significantly change the chemical composition of alfalfa hay. DM and AIA content increased in relation to process.

Interactions among treatment factors were generally nonsignificant. The only significant interactions were maturity-by-artificial rain level effect on available carbohydrate, lipids, cellulose and cell wall.

In general, alfalfa hay treated with artificial rain had a lower nutritive and economic value than when it is not affected by any rain.

(69 pages)

INTRODUCTION

Alfalfa has been recognized as a superior forage having good levels of high quality protein and digestible energy. It is used extensively in the United States to produce hay for livestock feeding especially for dairy cattle. A diet of alfalfa hay is generally satisfactory for maintenance of mature ruminants and horses. Lactating cows require about 1.5% of their body weight daily from fibrous feed for normal ruminal function. But high quality hay is preferred to maintain high milk production. Otherwise, lactating dairy cows require greater portions of energy feeds to supplement poorer quality forages used in the diet.

Nutritive value of hay can be significantly reduced unless harvest and curing are properly controlled. Sullivan (1973) observed that to produce a high quality hay, good forage must be harvested and dried with a minimum loss of nutrients. In order to preserve nutritive value, the hay producer must harvest at the proper stage of maturity and minimize damage from environmental conditions that can occur while the forage is drying.

The quality and quantity of the harvested field cured alfalfa hay depends on several factors that interact at the time of cutting and drying. Church (1978) indicated that the quality and quantity of field-cured hay can be attributed to the stage of maturity when cut, method of handling, moisture content and weather conditions during harvest, with leaching from rainfall generally considered to be the most destructive uncontrollable factor. Tukey (1970) pointed out that losses could result from oxidation, in the presence of air, enzyme activity or micro-organisms. Previous research has shown that carbohydrates, inorganic nutrients, protein and lipids are subject to chemical changes.

The main objectives of this research were to:

1. Determine chemical changes in alfalfa hay from artificial rain applied at various amounts and times during field drying process of alfalfa harvested at two stages of maturity.

2. Determine losses of yield of dry matter and nutrients from field-dried alfalfa treated with artificial rain.

REVIEW OF LITERATURE

Factors Affecting Quantity and Quality of Hay

Maturity of Forage

The stage of maturity of the forage at harvesting is a known factor influencing the chemical composition of alfalfa hay and other forages. Ely et al. (1953), for example, noted that immature grass has a higher protein content and higher feeding value than when it is cut at later stages of growth. Conrad et al. (1962) observed substantial changes in crude protein in grasses from very early stages through the latest stages of maturity. Also, he noted energy digestibility declines of 1 percent per day in some grasses in warmer climates.

Reid (1973) suggested that the best time to harvest alfalfa is at early flower (first flower to 1/10 bloom) when slightly more than half of the plant consists of leaves which contain most of the protein and vitamins. Some researchers have considered the best time of cutting alfalfa to be prior to 1/10 bloom or late vegetative stage. Wier et al. (1960) showed that yield of total digestible nutrients (TDN) and protein from alfalfa cut at four different stages of maturity were greatest when cutting at 1/10 bloom. But it was not the maximal point for digestibility of either TDN or protein. Therefore, nutrient value may be increased by cutting at early stages, although it should be noted that frequently cutting at a young stage can shorten the life of legumes such as alfalfa (Anderson et al., 1973; Reid, 1973).

Mac Donald (1946) suggested that although dry matter (DM) yield of the first cutting is reduced by advancing the cutting time, the seasonal yield of nutrients per area may be increased. The net energy increase from early cutting can be as much as 25%. Anderson (1976) also noted that a delay in cutting alfalfa brought an overall increase in total dry matter harvested. However, chemical data showed that this was due to an increase in the more fibrous parts of the plants. In general, Church (1978) concluded that as the plant matures, the protein content decreases, structural carbohydrates increase along with lignin, readily available carbohydrates decreases, and apparent digestibility of both protein and energy decreases.

Leaching during Field Curing

Field curing is commonly used to make alfalfa hay, but, as in other curing methods, the leafy portion of hay suffers from drying and handling more than stems. Leaching due to rain is one of the problems added to field curing. Heavy rain on dry hay can cause considerable leaching. Subsequent handling results in increased leaf shatter. Mecklenburg (1964) showed that leaves need only be wetted to be leached. It appears that leaves which have been wilted and dried are subsequently wilted more easily, indicating that intermittent rain tends to overcome the hydrophobic characteristics of some leaves. Those effects were noted by Shepherd et al. (1947), who found leaching losses from rain are less severe soon after mowing, but increase in severity during the curing process and with repeated showers. Slack et al. (1960) reported that alfalfa hay cured under rain conditions harvested 50 to 76% of the available DM vs. field curing without rain of 76%. Shepherd et al. (1954) reported DM losses of 36% when three scattered showers occurred during field curing. They also indicated that losses are higher for legumes than for grasses because of leaf shatter. Shepherd et al. (1954) specified some ranges of dry matter losses during field drying depending on the weather:

a)	respiration during wilting and drying	4 - 15%
b)	leaf shattering for grass	2 - 5%
c)	leaf shattering for legume hays	15 - 20%
d)	leaching by rain	5 - 14%

Anderson (1966) indicated an apparent lowering of soluble and more digestible portions of the alfalfa hay from rain including protein and nitrogen free extract (NFE) which results in an apparent increase in fiber. However, there are many factors associated with the intensity of the leaching effect. Shepherd et al. (1955) reported dry matter yield of all rain damaged field-cured alfalfa hay equal to 63.4% vs. 72.4% of all field cured alfalfa hay without rain damage. There was almost 10% greater dry matter losses in the rain-damaged hay.

An extensive review of leaching effects has been done by Tukey (1970) to point out several aspects of this phenomenon. Leaching as used in this review is defined as the removal of substances from plants by the action of aqueous solutions, such as rain, dew, mist and fog. However, all observations failed to demonstrate directly whether under proper conditions, many, if not all constituents, can be leached. Shepherd et al. (1955) noted high leaf protein and carotene losses in field-cured alfalfa hay under the effect of heavy rain. Voelker et al. (1970) showed that carotene content decreases with alfalfa drying and was reduced most under rain conditions. Field dry matter yield was lower in alfalfa hay under rainy conditions than during dry weather (53.6% vs. 76 to 86%, respectively).

Leaf, carotene and protein losses can be related to the shattering effect. It is known that in alfalfa, for example, 50% of the total weight of the plant is contained in the leaves, but the leaves contain 70% of the protein and 90% of the carotene content. Barnes and Gordon (1972) noted that it is essential to retain the leaves to make high quality hay.

Plant Cell Respiration

Some factors in hay processing are difficult to control. Loss of nutrients by cellular respiration of around 5% expected during field curing (Anderson, 1966). Carpintero et al. (1969) reported respiration losses of organic acid during wilting of alfalfa. Sullivan (1973) noted that the loss of carbohydrates in cut forage exposed to the air is mainly due to the respiration function of the plant. He explains that the rate of transpiration remains low for a few hours, depending on drying conditions, but there soon occurs a strong and pronounced increase that is associated with visible signs of wilting.

As the vacuole shrinks during drying, an inward pull is exerted on the protoplasm and an outward pull is exerted by the cell wall to which the protoplasm is attached. These tensions result in mechanical injury; then elimination of water becomes rapid and external factors control evaporation. In any case, carbohydrates seem to account for most of the dry matter losses during drying and wilting.

Drying Temperature

Although cool temperatures are more conducive to high quality forages during growth (Deinum et al. 1968, Deinum and Driven, 1972), warmer temperatures are generally desirable during curing. Wilkinson and Hall (1965) showed fresh immature alfalfa could loose as much as 4.5% of the dry matter per day when drying between 7 to 27^{o}C. Less loss occured at 27^{o}C and later stage of maturity. Church (1978) indicated that the faster the drying, the smaller the losses in nutritive value.

Ambient temperature and moisture content influence the generation of heat during the respiration of fresh cut herbage. Sullivan (1973) indicated that ambient temperature during drying has less effect on nitrogenous substances than on carbohydrates. But respiration losses of dry matter accompanied by the losses of the more soluble carbohydrates and cellulose constituents cause a greater loss in nutritive value. Temperature also affects loss of metabolites by leaching. Mitchell (1968) noted that carbohydrates are leached more easily at higher temperatures than at lower temperatures.

Nutrients and Chemical Analysis

The chemical characteristics and components of feedstuff that contribute to nutritive value have been studied for almost 200 years (Einhof, 1905 a,b). The history of this development has been documented by Tyler (1975). The deficiencies of the old proximate system analysis were discussed by Norman (1935). More recently, Van Soest and Wine (1967) presented his neutral detergent fiber technique attempting to separate cell wall constituents. While this method was an advance in forage analysis, Fonnesbeck and Harris (1970a) found difficulties in analyzing energy feeds, protein supplements and diets, or feces containing these ingredients, due to filtering and washing problems. Fonnesbeck and Harris (1973, 1976) proposed modifications to the chemical system for partitioning plant dry matter that shows certain advantages over the Van Soest system of analysis (Christiansen, 1979). Using their new system, all classes of feed can be accurately analyzed for cell walls (CW) and cell contents (CC) with the same procedure. Fonnesbeck (1976) reported the need for chemical analysis for all classes of feed and foods and suggested partitioning chemical components of feeds as they are utilized by animals. He specified common chemical properties of cell wall and cell content constituents, and grouped chemical components according to value as nutritive matter, partially nutritive matter and non-nutritive matter (see Figure 1).

New, more reliable methods for predicting DE from chemical analysis have been presented by Fonnesbeck et al. (1981b) where information provided by the diet description proved as useful as chemical analysis for the prediction of digestibility of nutrients.

Plant Cell Contents

Van Soest (1966) suggested that the cell contents represent the readily available nutritive matter. Fonnesbeck and Harris (1974) partitioned nutritive matter into protein, lipid and ash with soluble carbohydrates as the remainder.

Protein. Protein is another fraction of the cell content of the plant tissue, and its quality is measured by the ratio of indispensable amino acid and its efficient utilization by the animal. The more complete the assortment and the more nearly the proportions approach

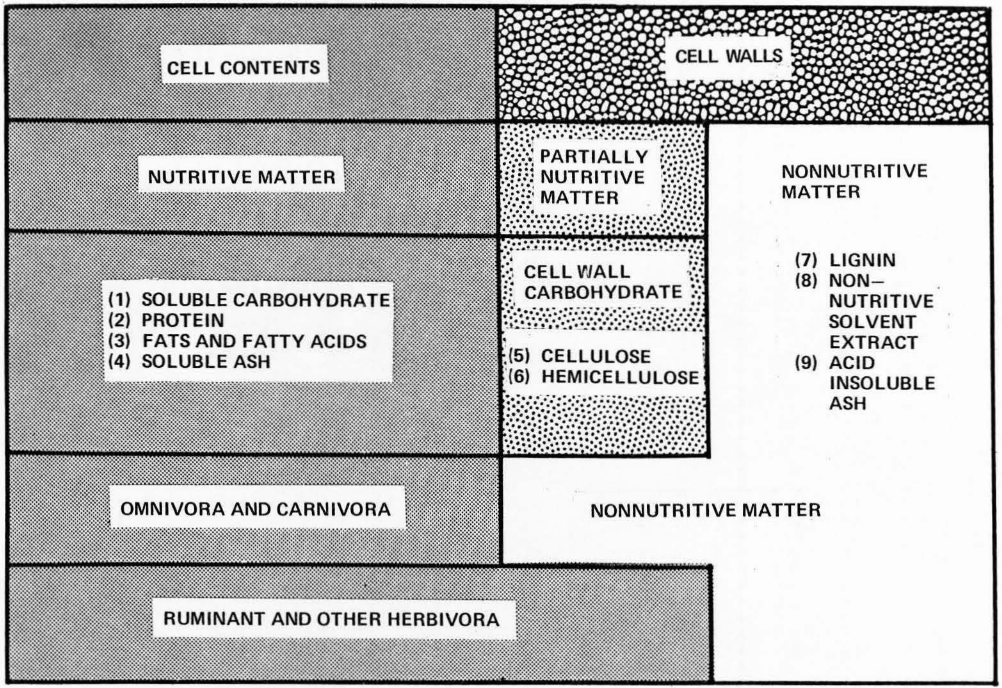


Figure 1. A realistic system for partitioning the chemical components of foods and feedstuffs. (Adapted from Harris 1970).

the physiological needs of an animal species for amino acid, the higher the quality of the protein (Lloyd et al., 1978). Protein is estimated in the plant and feedstuff by determining the nitrogen content. This nitrogen value is multiplied by the factor 6.25, because plant amino acids combined into proteins as a group consist of about 16% nitrogen.

Reid (1973) indicated that both legumes and non-legumes have their highest protein content at early growth, and that when hay is low in protein, it is also virtually low in digestible energy (DE). Anderson (1966) included protein with the soluble and more digestible portions of the plant apparently affected by rain. Reid (1973) indicated that most of the plant protein is found in leaves. Terry and Tilley (1964) reported that leaf blades, leaf sheaths, and stems in grass were of high digestibility. Ely et al. (1953) showed also the result of decreasing values of protein in orchard grass because of maturity.

There is a non-protein nitrogen content in the plant which has nearly the same nutritive value as true protein depending on animal specie. This portion can also be influenced by weather stress, plant maturity and specie. In an example (Brady, 1960) noted that total nitrogen of ryegrass dropped from 2.02 to 1.83% in 2.5 hr of wilting; the proportion of non-protein nitrogen was 8.9 to 11.4% of the total nitrogen and 2.6 to 5.9% was free amino nitrogen.

Lipids. Lipids represent another part of the plant cell content. Lipids are considered a high source of energy, with about 2.25 times more energy than carbohydrates. Generally, they are found in the leaf tissue in the range of 3-10% declining with maturity.

Some lipids are linked with plant protein and carbohydrates (Folch 1951, Fonnesbeck 1973, Hoggenraad et al., 1970, Hannahan, 1960). Lipids are usually represented by the ether extract (EE) fraction in the proximate analysis. But many authors, such as Bligh and Dryer (1959) and Hanahan (1960), have investigated chemical procedures to efficiently extract all lipids. It has been found that a 2:1 chloroform:methanol extraction at room temperature extracted 100% of the lipids. This procedure has been adopted by Fonnesbeck and Harris (1974) to analyze feed and foods and is suggested as a replacement for the ether extract procedure.

Fonnesbeck and Harris (1974) published a solvent extract procedure for determining total lipids and for separating them into nutritive and non-nutritive lipids. Non-nutritive lipids of the leaves and stems usually exceeded the nutritive portion. The nutritive lipid fraction fraction was considerably less than ether extract and ether extract method shown to be less efficient than solvent extract.

Soluble minerals. Minerals are another portion of the food which represent the inorganic nutrient of plants. It is known that minerals are concentrated in the leaves and are highly affected by leaching. Mann and Wallace (1925) were the first to use the term leaching to describe the removal of mineral nutrients from leaves soaked in water. Lutwick and DeLong (1954) extracted soluble materials from decomposing leaves by rain. Tukey (1970) mentioned Ca, K, Mg and Mn as the main nutrient minerals which can be leached from plant tissue.

Ash analysis represents the inorganic portion or mineral content of feed which is estimated in the laboratory by total incineration and oxidation of the organic matter (OM) of the plant. Fonnesbeck and

Harris (1970b) found that the ash remaining in the crucible after determining plant cell wall, hemicellulose and lignin by their method, could be reported as acid insoluble ash (AIA) or silica. Fonnesbeck (1976) showed that soluble ash content and organic cell content (OCC) is determined by subtracting soluble ash from cell content (CC). Total cell content can be calcualted by subtracting cell wall from 100% plant dry matter.

Available Carbohydrates. Sugar and starches are found in the plant cell content representing the portion of the carbohydrates most soluble in water and available to the animal. They are the chief components of the highly energetic digestible cell content (Lloyd et al., 1978). Van Soest (1963a, 1963b, 1967) showed that detergent solution treatment results in the extraction of plant cell content constituents (lipids, sugars, organic acids, non-protein pectins, soluble proteins, etc.). Fonnesbeck (1976) indicated various regression equations for calculating digestible energy (DE) which were highly correlated to soluble carbohydrates, sugars and starches content, although those predictions may not be valid for all feeds.

Plant Cell Walls

Fonnesbeck (1976) suggests that the nutritive quality of feeds can be given based on chemical evaluation. The cell wall fraction is a combination of partially nutritive and non-nutritive matter and is negatively correlated to digestible energy (DE) for the species in general. Minish and Fox (1979) wrote that the higher the proportion of cell wall the lower the total digestible nutrient (TDN) and net energy (NE) value of the feed.

Cellulose, hemicellulose, lignin and pectins of the plant cell wall constitute the coarse fibrous fraction of the feed. Schneider and Flatt (1975) wrote that the crude fiber, determined by proximate analysis, indicates the coarseness of a forage or feedstuff.

Cellulose and hemicellulose are the most abundant structural carbohydrates in the plant cell walls. Cellulose is more digestible when plants are at an immature stage of growth, but digestibility decreases as the plant becomes older because older plants contain more indigestible lignin decreasing energy value (Minish and Fox, 1979). Maynard et al. (1979) defines hemicellulose as a carbohydrate soluble in mild alkali and less resistant to chemical degradation than cellulose. Van Soest (1966) reported hemicellulose as a partially digestible fraction. Hungate (1966) noted that the fibrous portion of plant is digested by digestive microbial enzymes of animals.

While lignin is a class of non-carbohydrate compound which gives structural support to plant cell walls, lignin is the indigestible portion of the plant which also reduces the cellulose and hemicellulose digestibility.

Pectin is found primarily in the spaces between plant cell walls. Pectin digestibility depends entirely on microbial action.

The partitioning of plant dry matter into neutral detergent fiber (NDF) has been presented as an accurate separation of cell wall constituents and the readily soluble portion contained within the plant cell (Van Soest, 1963a; 1963b; Van Soest and Marcus, 1964; Van Soest and Moore, 1965; Van Soest, 1965).

Christiansen (1979) reported a further improvement of the chemical procedures by Fonnesbeck and Harris (1970a,b) over the NDF procedure of

Van Soest. Fonnesbeck and Harris (1970a) found that protein was the main cause of the filtering problems encountered in the NDF procedure with energy feeds. The improved method can be used in analyzing energy feeds, protein feeds and fibrous feeds without filtering problems and by simplified laboratory methods (Fonnesbeck and Harris, 1970a, b, 1974). As Fonnesbeck (1976) reported, crude fiber values are substantially lower than cell wall values for feeds and foods having considerable hemicellulose in the cell wall because CW by this method includes the hemicellulose, lignin and silica lost in the crude fiber analysis (Norman, 1935).

METHOD AND PROCEDURE

Design of Experiment

Approximately 3.2 hectares of alfalfa were divided, swathed and treated with artificial rain according to a 2 x 3 x 2 factorial arrangement experiment. Factors were 2 stages of maturity of alfalfa (1 = late vegetative; 2 = early bloom), 3 levels of artificial rain applied (1 = no rain; 2 = low or approximately 5mm; 3 = high or approximately 20mm), and 2 times of applying artificial rain (1 = 21 hours after cutting when the forage was 40-60% dry matter; 2 = 45 hours after cutting when the forage was 60-75% dry matter). The experimental design is outlined in Table 1.

Hay Processing Methods

A contract was made with a private owner to harvest and purchase the second cutting of alfalfa from a 3.2 ha plot located in North Logan, Cache County, 2 miles from Utah State University campus. The alfalfa crop was swathed using a 3.05 meters wide, 489 New Holland swather powered by a 5600 Ford tractor. Hay was partially field cured and treated with water using a hand line sprinkling irrigation system. Sprinkling pipes, 9.14 meters (30 ft) long, were placed between two swaths. Two swaths on each side of the line were totally sprinkled. The third swath rows was only partially wetted by the sprinkle and this hay was discarded (see Figure 2).

Table 1. Factorial arrangement of treatments for experimental alfalfa hay[a].

	Sprinkling Time and Artificial Rain Levels Applied						
	T_1			T_2			
Stage of Maturity	C	L	H	C	L	H	Totals
Late vegetative	1[b]	2	3	4	5	6	6
Early bloom	7	8	9	10	11	12	6
Totals	2	2	2	2	2	2	12

[a]T_1 = sprinkling time when alfalfa was about 40 to 60% dry matter; T_2 = sprinking time when alfalfa was about 60 to 75% dry matter; C = control no water applied; L = low level of water applied by sprinkling for 1 hour; H = high level of water applied by sprinkling for 4 hours.

[b]Treatment number.

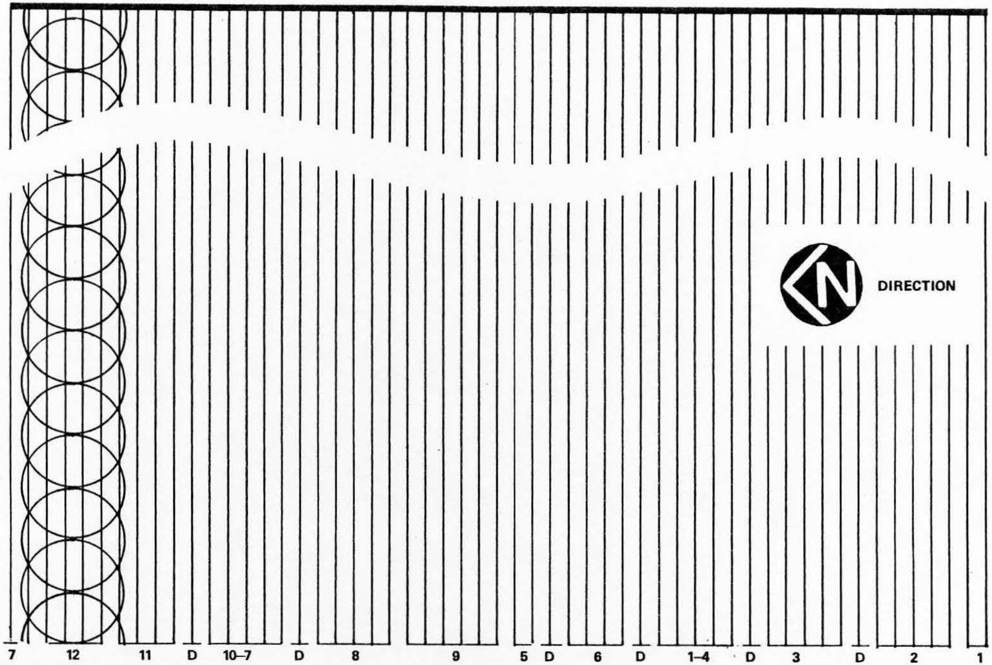


Figure 2. Location of alfalfa treatments in experimental plot. One line of the sprinkling irrigation system is illustrated. (Field dimensions: 167 meters wide, 201 meters long.)

Control alfalfa swath rows without sprinkling rain were left for each specific area. The low level of water applied was sprinkled for one hour and the high level was sprinkled for four hours.

Rain gages made with funnels and graduated cylinders were placed under the irrigated sprinkling area to measure the levels of water applied at both times. However, it was impossible to measure a consistent level of sprinkled water because the sprinkling nozzels did not distribute the water uniformily over the area. Therefore, water application was regulated by a timed period with constant pressure. The water pressure from a gravity pressurizied irrigation system was 1.7 Kg/cm^2 (24 psi). The sprinkling heads were 9.14 meters (30 ft) apart with nozzel diameter of 0.40 cm (10/64 inch). The delivered amount of water in the sprinkling irrigated area was thus calculated to be about 4.86 mm per 1 hour and 19.44 mm in 4 hours.

Time of baling was determined by the condition of the hay in all the swaths, during the morning. Most of the forages were packaged in bales about 29 Kg with a 269 New Holland baler powered by a 4000 Ford tractor. Each bale was labeled by treatment number. Two of three days after baling the hay bales were hauled and stocked under shelter.

Sampling Procedure

The 3.2 hectare of second crop alfalfa was divided in half to be harvested at two different stages of maturity. Treatments 1 to 6 were harvested at late vegetative stage of maturity while treatment 7 to 12 were cut at the early bloom stage. Alfalfa samples of the complete twelve treatments and the corresponding fresh and pre-sprinkling control samples were collected, weighed and dried. Samples from fresh cut alfalfa were collected at random in different sections of the field. They were immediately packaged and sealed in plastic bags with dry ice to reduce evaporation and respiration losses. The samples were placed in an insulated box for transportation to the laboratory. Before weighing, dry ice was removed from the sample and the plastic bag was sealed again to retain fresh sample initial moisture content.

Samples were stored and frozen to preserve initial nutrients. Thereafter, fresh samples were either freeze dried or microwave oven dried and equilibrated to atmospheric air moisture in paper bags. The weight of the air dry sample and the fresh sample was used to calculate the partial dry matter content of the fresh alfalfa forage.

After 21 hours after cutting, half of the alfalfa swaths were sampled as pre-sprinkling T_1 or 40-60% dry matter. The samples were collected in plastic bags, sealed and stored in an insulated box to protect them from dehydration. Samples were immediately taken to the laboratory to be weighed and air dried in paper bags until air equilibrium, and then finally ground.

Alfalfa samples of 60-75% dry matter were collected at about 45 hours after cutting. They were collected from the other half of alfalfa swaths. Samples were called pre-sprinkling T_2, and were processed in the same manner as the previous samples.

Pre-baling samples were taken from swaths treated at different levels of water applied. They were collected just before the baler picked up the swath. Samples were weighed, dried, and data recorded at the laboratory, using the same procedure as the other samples.

Pre-feeding samples were collected after hay was baled, handled, stored and chopped to be offered to animals at subsequent feeding and digestion trials.

Fresh, pre-sprinkling, pre-baling and pre-feeding samples were ground with a Willey mill and 1 mm screen in preparation for chemical analysis.

Yield Measurement

Dry matter yield per treatment of different processing times was recorded during collection. A 3.048 x 3.048 meter plastic sheet (10 x 10 ft) and a scale (lb) were used to collect and record weight of fresh cut alfalfa from 9.29 m^2 (100 ft^2) surface area. Between 3 and 10 samples were collected and weighed for each treatment area in the alfalfa plot. The dry matter yield data was estimated in Kg/ha using a conversion factor of 488.24 to convert pounds per 100 ft^2 to kilograms per hectare.

Weather Observations

Alfalfa hay was processed from July 28, 1980 to August 8, 1980. A portable weather station was placed in the middle of the field to obtain temperature, relative humidity, and wind velocity over the alfalfa during the whole processing period. Temperature and relative humidity was also measured from under alfalfa swaths. The station was a standard ventilated instrument shelter containing the instruments to measure weather conditions. Weather conditions were recorded at least once daily through the whole processing period, and at the end time when alfalfa was cut, pre-sprinkled, sprinkled, and baled.

Wind velocity was measured by a 3 cup anemometer with a minimum threshold of 3 miles per hour. The data were recorded on the scale of miles/hr and recalculated to Km/hr. Relative humidity was measured by a portable, electrically aspirated psychometer manufactured by Bendix Environmental Science Division, model 566-2 (Fahrenheit temperature). The relative humidity of the air over and under the hay swath was measured, and the data was recorded as a percentage of saturation. The ambient temperature was measured by a Taylor #5458 maximum-minimum (low) self-registering thermometer (Farhenheit scale). The data were subsequently transformed to the celsius scale.

Chemical Analysis

Samples were analyzed for dry matter, nitrogen, ash, plant cell walls, cellulose, hemicellulose, lignin, total lipids, and acid insoluble ash. Available carbohydrate and soluble ash were calculated.

The Fonnesbeck and Harris modifications of the Van Soest method (1970a) was used to obtain maximum recovery of the cell wall constituents with maximum removal of the cell contents. The procedure is summarized in Figure 3.

Hemicellulose was separated from cell wall residue using a 4% sulfuric acid solution (Fonnesbeck and Harris, 1970b). The cellulose portion of cell walls was calculated by difference following a 3 hour digestion with 72% sulfuric acid on the 4% sulfuric acid residue. The lignin component of plant cell walls was calculated by difference after the 72% sulfuric acid residue had been ashed. This residue represents the acid insoluble ash of the total feed, primarily silica.

1 g of air-dry sample

Fonnesbeck and Harris modification of Van Soest method

Maximum removal of the cell' contents

Cell walls residue

4% H_2SO_4 at boiling temperature for 1 hour to hydrolyze hemicellulose

Hemicellulose

Cellulose, lignin, acid insoluble ash

72% H_2SO_4 at room temperature for 3 hours

Cellulose

Lignin, acid insoluble ash

500^{o} C in furnace

Organic matter

Acid Insoluble Ash

Figure 3. Flow diagram for sequential analysis of Fonnesbeck and Harris modification of Van Soest method of neutral detergent fiber.

Soluble ash or nutritive ash portion was calculated by subtracting acid insoluble ash from the total ash fraction (Fonnesbeck, 1976).

The technique of cloroform:methanol extraction procedure by Fonnesbeck (1974) was used to determine total lipids to replace the inaccurate ether extract (EE) determination of the proximate system of analysis. The quantity of available carbohydrates was calculated as cell contents minus protein minus total lipids minus soluble ash. Dry matter and protein were determined using procedures outlined by Harris (1970). Gross energy was determined with a Parr bomb calorimeter.

Statistical Analysis

A factorial experiment design was selected to analyze the yield response (Y) produced by each factor or independent variable (stage of maturity, artificial rain, sprinkling time and process). The twelve treatments that were applied to the experimental unit generated the factor level combinations. Analysis of variance (AOV) was performed to examine the effect of each factor singly, and in each possible two way interaction combination. Although analytical precision is increased by separating interaction effects from error effects (Snedecor, 1956), it was felt that the small sample size precluded accurate analysis of higher order interactions. Thus, the results reported here assume that three and four way interactions are negligible and as a result they are combined with the error term. Fisher's least significant different (LSD) procedure was used to determine which population means differ after we rejected the hypothesis of equality of t population mean in an AOV. Analysis of variance and regression analysis were computed using Rummage II by Scott et al. (1981) at the Utah State University Computer Center.

RESULTS AND DISCUSSION

Yield

The dry matter yield and chemical composition of alfalfa at cutting and pre-sprinkling are given in Table 2.

Since each treatment was grown on a slightly different area and the field was not as uniform as desired, differences in fresh and pre-sprinkled yield observations probably reflect the variability of the alfalfa stand of the field. The highest yield should be obtained from the fresh cut observations, but it was not possible to produce duplicate observations from the same area.

Changes in dry matter yield from fresh to pre-baling field cured alfalfa hay were not consistent. However, it could be noted that yield of fresh alfalfa increased from 2,724 Kg/ha to 3,206 Kg/ha when alfalfa was at late vegetative and early bloom maturity, respectively. This result is in agreement with Wier et al. (1960) and Moline et al. (1962) who showed continued increment of dry matter yield with advance in maturity of alfalfa.

The inconsistent yield of dry matter among treatments was also reflected in the yield of nutrients. The large experimental error marked any statistically significant differences of yield of nutrients. Results were probably due to the difference among sampling areas assigned to the different treatments in the experimental plot. Based on this, there is a definite need to carry this type of experiments on more uniform plots with more replications per treatments.

Table 2. Dry matter yield and chemical composition (dry basis) of fresh cut alfalfa forage and drying alfalfa just before artificial rain was applied.

Time of Sampling	Description: Maturity	Description: Time of Sprinkling[a]	Dry Matter yield kg/ha	Dry Matter %	Ash %	Gross Energy Mcal/kg	Plant Cell Contents %	Crude Protein (N x 6.25) %	Available Carbohydrates %	Soluble Ash %	Total Lipids %
Fresh	Late veg	--	2,724	21.83	9.84	4.37	61.86	17.96	29.15	9.39	5.37
Pre-sprinkling		1	3,000	46.97	9.34	4.34	60.11	17.01	29.05	8.82	5.23
		2	2,951	69.23	10.16	4.35	61.85	19.59	26.86	9.76	6.64
Pre-baling control hay		--	3,041	84.96	9.83	4.31	63.92	18.25	30.74	9.54	6.51
Fresh	Early bl	--	3,206	24.00	9.50	4.21	59.65	18.18	26.67	9.08	5.73
Pre-sprinkling		1	3,218	61.32	9.47	4.38	57.18	17.23	25.44	9.29	5.22
		2	3,366	71.08	9.64	4.37	58.54	16.15	27.07	9.55	5.77
Pre-baling control hay		--	3,221	86.46	9.22	4.31	58.27	18.30	25.23	8.85	5.89

Time of Sampling	Description: Maturity	Description: Time of Sprinkling[a]	Cell Walls %	Cellulose %	Hemicellulose %	Lignin %	Acid Insoluble Ash %
Fresh	Late veg	--	38.14	23.69	7.97	6.69	0.46
Pre-sprinkling		1	39.89	25.33	7.09	7.05	0.52
		2	38.15	24.09	6.43	6.49	0.40
Pre-baling control hay		--	36.08	22.35	7.03	6.42	0.29
Fresh	Early bl	--	40.35	25.59	8.51	7.15	0.40
Pre-sprinkling		1	42.82	27.07	7.80	7.77	0.18
		2	41.46	26.50	6.62	8.24	0.10
Pre-baling control hay		--	41.74	25.68	8.22	7.15	0.37

[a]Alfalfa was sampled before sprinkling time 1, when alfalfa was about 40 to 60% dry matter; alfalfa was sampled before sprinkling time 2, when alfalfa was about 60 to 75% dry matter.

Table 3. Analysis of variance and mean squares of chemical composition (dry basis) of non-sprinkled alfalfa during the drying process.

Source	df	Dry Matter %	Ash %	Gross Energy Mcal/kg	Plant Cell Contents: Crude Protein (N x 6.25) %	Available Carbohydrates %	Soluble Ash %	Total Lipids %
Maturity (M)	1	38.772	0.765	0.011	1.415	19.102	0.533	1.068*
Sprinkling time (T)	1	93.800	0.004	0.000	0.024	0.219	0.011	0.264
Process (P)[a]	2	920.119*	0.060	0.003	0.644	1.912	0.348	0.118
MT	1	36.575	0.039	0.002	0.034	0.145	0.015	0.128
MP	2	15.245	0.075	0.019	0.721	6.719	0.228	1.352*
TP	2	81.553	0.306	0.000	0.789	1.459	0.482	0.352
Error	2	10.169	0.033	0.002	3.969	7.544	0.058	0.046

Source	df	Cell Walls %	Cell Wall Constituents: Cellulose %	Hemicellulose %	Lignin %	Acid Insoluble Ash %
Maturity (M)	1	53.636***	25.608***	0.350	2.279*	0.018
Sprinkling time (T)	1	0.517	0.205	0.378	0.069	0.001
Process (P)[a]	2	2.811	3.739*	0.409	0.368	0.132
MT	1	0.460	0.001	0.476	0.279	0.005
MP	2	1.683	0.538	0.814	0.100	0.046
TP	2	0.989	0.314	0.245	0.092	0.018
Error	2	0.273	0.135	0.854	0.073	0.008

[a]Samples were collected, pre-sprinkled, pre-baling and pre-feeding.

*P<.05

**P<.01

***P<.001

Chemical Composition of Fresh Alfalfa Forage and Unsprinkled Alfalfa Hay

Chemical composition of fresh alfalfa forage and unsprinkled alfalfa hay is shown in Table 2 with the analysis of variance of the chemical composition shown in Table 3.

The difference in dry matter content between fresh and partially dried alfalfa forage is an obvious effect of the method of selecting samples. There was a 2 percent increase in dry matter content of fresh alfalfa forage with advancing maturity (Table 2). Dry matter percent of fresh alfalfa at late vegetative was 21.83% compared to 24.0% at early bloom, but this was not a statistically significant difference.

Plant cell contents percentages were slightly lower in early bloom, and plant cell walls were higher at early bloom than in the late vegetative stage of maturity. These results agree with Goering et al. (1976) and Anderson (1976), who noted that cell walls increased with advancing maturity in alfalfa. Cellulose and cell wall were significantly ($P<.001$) increased and lignin was increased ($P<.05$) under the maturity factor effect. Cellulose percentage was significantly ($P<.05$) increased from fresh to pre-sprinkling and pre-baling control. Cellulose was increased significantly ($P<.001$) by stage of maturity. Protein and available carbohydrates showed slight change at different stage of maturity. Protein percentage decreased from 18.2% at late vegetative to 17.2% averaged at early bloom. Also, averabe available carbohydrates percentage decreased from 28% to 26.40% at early bloom. However, these changes were not statistically significant. Anderson (1976) and Moline (1962) noted a decrease in percent crude protein with advances in maturity of alfalfa. Blaser (1964) found that soluble carbohydrates in alfalfa decrease under the influence of stage of growth, but Fonnesbeck et al. (1981) found available carbohydrates of alfalfa hay increased with maturity in their experiment. Ash, soluble ash, total lipids, and gross energy values remained quite similar at both processing times and stages of maturity, and no significant difference was noted except lipids showing significant ($P<.05$) effect of maturity and maturity x process interaction (Table 3).

Chemical Composition of Alfalfa Hay

Results of chemical analysis of twelve different treatments of alfalfa hay are shown in Tables 4, 5, and 7, and the corresponding statistical analyses are shown in Table 6.

Maturity. The more fibrous part of the plant constituents increase, decreasing protein and the total fraction of the plant cell content (Weir et al., 1960; Anderson, 1976; Goering et al., 1976). Fonnesbeck et al. (1981) noted that as the alfalfa hay matured, cell wall (CW) constituents increased only slightly, but available carbohydrates (AC) content increased while crude protein and soluble ash decreased.

The dry matter (DM) content of the hay was not significantly different among treatments means except for the difference between pre-baling and pre-feeding. The higher water content was required at baling to optimize recovery of alfalfa.

Higher ash content was found at late vegetative than at early bloom stage of maturity (Table 5). The average ash content were 9.62% vs. 8.24% at late vegetative and early bloom respectively.

Table 4. Dry matter yield and chemical composition (dry basis) of alfalfa hay at time of pre-baling and before feeding.

Treatment	Description: Maturity	Description: Amount of Water Applied	Description: Time of Sprinkling[a]	Time of Sampling	Dry Matter Yield Kg/ha	Dry Matter %	Ash %	Gross Energy Mcal/kg	Cell Contents %	Crude Protein %	Available Carbohydrates %	Soluble Ash %	Total Lipids %
1	Late veg	C	1	Pre-baling	3,041	82.47	10.12	4.27	63.58	19.84	28.41	9.99	5.36
2		L	1		2,454	91.39	9.67	4.12	58.09	18.44	25.11	9.63	4.91
3		H	1		3,664	83.64	10.19	4.35	57.77	18.20	25.18	9.68	4.71
4		C	2		3,041	87.46	9.53	4.34	64.26	16.65	33.07	9.08	5.46
5		L	2		2,756	83.93	9.30	4.30	57.30	18.02	25.09	9.14	5.05
6		H	2		2,677	87.74	9.26	4.34	55.02	18.44	23.55	8.72	4.31
1		C	1	Pre-feeding		89.88	10.12	4.45	62.11	17.94	27.16	10.12	6.89
2		L	1			91.10	9.56	4.45	60.92	19.14	26.35	9.56	5.87
3		H	1			90.63	8.96	4.41	57.08	18.16	24.68	8.85	4.59
4		C	2			89.88	10.12	4.45	62.11	17.94	27.16	10.12	6.89
5		L	2			91.00	9.31	4.37	54.82	18.41	21.64	9.23	5.54
6		H	2			91.23	9.34	4.33	59.47	20.00	25.69	9.34	4.44
7	Early bl	C	1	Pre-baling	3,221	88.19	9.53	4.35	58.91	17.73	25.97	9.18	6.03
8		L	1		3,534	86.28	9.61	4.36	61.33	16.73	30.13	9.19	5.28
9		H	1		3,044	85.98	8.76	4.31	56.28	18.27	23.96	8.50	5.55
10		C	2		3,221	84.73	8.90	4.27	57.62	18.86	24.49	8.52	5.75
11		L	2		3,463	81.17	7.84	4.41	55.36	16.62	24.59	7.74	6.41
12		H	2		3,128	79.09	9.03	4.31	55.69	17.50	23.33	9.03	5.83
7		C	1	Pre-feeding		91.07	9.41	4.23	58.20	17.43	26.30	9.41	5.06
8		L	1			91.61	8.20	4.17	59.36	16.65	28.49	7.99	6.23
9		H	1			90.98	9.30	4.39	59.07	18.69	25.72	9.05	5.61
10		C	2			91.07	9.41	4.23	58.20	17.43	26.30	9.41	5.06
11		L	2			91.36	8.35	4.31	56.80	18.20	25.58	8.12	4.90
12		H	2			92.03	8.65	4.40	56.67	15.89	27.40	8.53	4.85

[a]Sprinkling time 1 when alfalfa was about 40 to 60% dry matter.

[a]Sprinkling time 2 when alfalfa was about 60 to 75% dry matter.

C: Control no water applied.

L: Low level of water applied by sprinkling for 1 hour.

H: High level of water applied by sprinkling for 4 hours.

RVI: Relative value index.

Table 4. (Continued)

Treat-ment	Description: Maturity	Description: Amount of Water Applied	Description: Time of Sprink-ling[a]	Time of Sampling	Dry Matter Yield Kg/ha	Dry Matter %	Cell Walls %	Cellu-lose %	Hemi-cellu-lose %	Lignin %	Acid Insoluble Ash %	RVI
1	Late veg	C	1	Pre-baling	3,041	82.47	36.42	22.09	7.83	6.37	0.13	1.086
2		L	1		2,454	91.39	41.91	26.59	7.12	8.20	0.04	1.019
3		H	1		3,664	83.64	42.23	26.29	7.32	7.12	0.51	1.019
4		C	2		3,041	87.46	35.74	22.61	6.23	6.47	0.44	---
5		L	2		2,756	83.93	42.70	27.40	7.57	7.66	0.15	1.000
6		H	2		2,677	87.74	44.98	29.18	7.02	7.51	0.54	0.981
1		C	1	Pre-feeding		89.88	37.89	23.94	7.65	6.82	0.00	1.058
2		L	1			91.10	39.08	25.21	6.63	7.23	0.00	1.053
3		H	1			90.63	42.92	27.70	7.36	8.18	0.11	1.000
4		C	2			89.88	37.89	23.94	7.65	6.82	0.00	1.058
5		L	2			91.00	45.18	28.98	7.01	8.29	0.08	0.967
6		H	2			91.23	40.53	25.91	6.86	7.77	0.00	1.047
7	Early bl	C	1	Pre-baling	3,221	88.19	41.09	25.82	7.56	6.70	0.36	1.019
8		L	1		3,534	86.28	38.67	24.58	7.39	6.88	0.42	1.025
9		H	1		3,044	85.98	43.72	26.42	8.80	8.24	0.26	1.000
10		C	2		3,221	84.73	42.38	25.54	8.87	7.60	0.38	1.014
11		L	2		3,463	81.17	44.64	29.48	8.44	7.98	0.10	0.986
12		H	2		3,128	79.09	44.31	28.36	8.08	7.88	0.00	1.000
7		C	1	Pre-feeding		91.07	41.80	27.30	7.04	7.47	0.00	1.006
8		L	1			91.61	40.64	26.07	7.19	7.18	0.21	1.006
9		H	1			90.98	40.93	25.25	7.83	7.62	0.25	1.033
10		C	2			91.07	41.80	27.30	7.04	7.47	0.00	1.006
11		L	2			91.36	43.20	27.61	6.88	8.49	0.23	1.000
12		H	2			92.03	43.33	27.14	7.69	8.39	0.12	0.972

[a]Sprinkling time 1 when alfalfa was about 40 to 60% dry matter.

[a]Sprinkling time 2 when alfalfa was about 60 to 75% dry matter.

C: Control no water applied.

L: Low level of water applied by sprinkling for 1 hour.

H: High level of water applied by sprinkling for 4 hours.

Table 5. Chemical composition (dry basis) of alfalfa hay by treatment[a].

					Plant Cell Contents									
Treatment Number	Treatment Description[b]	Dry Matter %	Ash %	Gross Energy Mcal/kg	Crude Protein (N x 6.25) %	Available Carbohy-drates %	Soluble Ash %	Total Lipids %	Cell Walls %	Cell-ulose %	Hemi-cell-ulose %	Lignin %	Acid Insoluble Ash %	RVI
1	$M_1 W_1 T_1$	86.18	10.12	4.36	18.89	27.79	10.06	6.13	37.16	23.02	7.74	6.60	0.07	1.072
2	$M_1 W_2 T_1$	91.25	9.62	4.28	18.79	25.73	9.60	5.39	40.50	25.90	6.88	7.72	0.02	1.033
3	$M_1 W_3 T_1$	87.14	9.58	4.38	18.18	24.93	9.27	4.65	42.58	27.35	7.34	4.65	0.31	1.000
4	$M_1 W_1 T_2$	88.67	9.83	4.39	17.30	30.12	9.60	6.18	36.82	23.28	6.94	6.65	0.22	1.044
5	$M_1 W_2 T_2$	87.47	9.31	4.33	18.22	23.34	9.19	5.30	43.94	28.19	7.29	7.98	0.12	1.000
6	$M_1 W_3 T_2$	89.49	9.30	4.33	19.22	24.62	9.03	4.38	42.76	29.55	6.94	7.64	0.27	1.014
7	$M_2 W_1 T_1$	89.63	9.47	4.29	17.58	26.14	9.30	5.52	41.45	26.56	7.30	7.09	0.18	7.019
8	$M_2 W_2 T_1$	88.95	8.91	4.26	16.69	29.31	8.59	5.76	39.66	25.33	7.29	7.03	0.32	1.025
9	$M_2 W_3 T_1$	88.48	9.03	4.35	18.48	24.84	8.78	5.58	42.33	25.84	8.32	7.93	0.26	1.019
10	$M_2 W_1 T_2$	87.90	9.16	4.25	18.15	25.40	8.97	5.41	42.09	26.42	7.96	7.54	0.19	1.019
11	$M_2 W_2 T_2$	86.27	8.10	4.36	17.41	25.09	7.93	5.66	43.92	28.55	7.66	8.24	0.17	0.986
12	$M_2 W_3 T_2$	85.56	8.84	4.36	16.70	25.37	8.78	5.34	43.82	27.75	7.89	8.14	0.06	0.986

[a]Treatment means.

[b]M_1 = late vegetative; M_2 = early bloom; W_1 = no water applied; W_2 = low level water applied; W_3 = high level water applied; T_1 = sprinkling time when alfalfa was about 40 to 60% dry matter; T_2 = sprinkling time when slfalfa was about 60 to 75% dry matter; RVI = Relative value index.

Table 6. Analysis of variance and mean squares of chemical composition (dry basis) of alfalfa hay.

					Plant Cell Contents			
Source	df	Dry Matter %	Ash %	Gross Energy Mcal/kg	Crude Protein (N x 6.25) %	Available Carbohy-drates %	Soluble Ash %	Total Lipids %
Maturity (M)[a]	1	1.921	11.509*	0.0079	5.189	0.029	3.219***	0.269
Water time (W)[b]	2	6.179	7.029	0.0016	0.046	15.240*	1.124*	0.892
Sprinkling time (T)[c]	1	1.038	2.470	0.0051	0.034	0.767	0.009	0.836
Process (P)[d]	1	202.827***	2.112	0.0870	0.015	0.007	0.074	0.068
MT	1	1.820	1.675	0.0014	1.335	9.920	0.451	0.160
MW	2	7.636	3.297	0.0086	1.038	14.694*	0.002	1.179*
MP	1	10.127	2.968	0.0420*	0.493	9.438	0.016	2.381**
TW	2	5.293	1.624	0.0065	0.612	7.781	0.275	0.174
TP	1	0.878	3.650	0.0006	0.002	2.130	0.204	0.109
WP	2	10.333	3.667	0.0016	0.621	2.497	0.463	0.504
Error	9	6.166	2.239	0.0069	1.269	2.908	0.173	0.245

			Cell Wall Constituents			
Source	df	Cell Walls %	Cell-ulose %	Hemi-cell-ulose %	Lignin %	Acid Insoluble Ash %
Maturity (M)	1	15.105*	4.446	55.69	0.499	0.0045
Water (W)	2	35.969***	20.610***	36.99	2.154***	0.0122
Sprinkling time (T)	1	2.368	0.067	43.85	0.135	0.0350
Process (P)	1	0.540	0.069	52.22	0.406	0.2262**
MT	1	1.179	0.368	51.57	0.427	0.0950*
MW	2	15.890*	10.131***	39.26	0.410	0.0203
MP	1	0.286	0.005	52.63	0.008	0.0345
TW	2	4.892	1.444	33.95	0.217	0.0042
TP	1	2.884	1.046	33.56	0.003	0.0392
WP	2	2.361	3.977	40.84	0.158	0.0295
Error	9	2.224	0.935	35.27	0.183	0.0178

[a]Cut at late vegetative versus early bloom stage of maturity.

[b]Low level and high level of water were applied by sprinkling for 1 hour and 4 hours, respectively.

[c]The sprinkling time 1 and 2 were when alfalfa hay was between 40-60% DM or 60-75% DM, respectively.

[d]Alfalfa hay samples were collected before baling and before feeding.

*P <.05

**P <.01

***P <.001

Table 7. Chemical composition (dry basis) of alfalfa hay under different harvesting conditions[a].

Factor Levels	Dry Matter %	Ash %	Gross Energy Mcal/kg	Plant Cell Contents: Crude Protein (N x 6.25) %	Plant Cell Contents: Available Carbohydrates %	Plant Cell Contents: Soluble Ash %	Plant Cell Contents: Total Lipids %	Cell Walls %	Cellulose %	Hemicellulose %	Lignin %	Acid Insoluble Ash %
Maturity (M)												
Late vegetative	88.37^{b}	9.62^{b}	4.35^{b}	18.43^{b}	26.10^{b}	9.46^{g}	5.34^{b}	40.62^{b}	25.88^{b}	7.19^{b}	7.37^{b}	0.17^{b}
Early bloom	87.80^{b}	8.92^{b}	4.31^{b}	17.50^{b}	26.02^{b}	8.72^{h}	5.55^{b}	42.21^{c}	26.74^{b}	7.74^{b}	7.66^{b}	0.19^{b}
Artificial Rain (W)												
0	88.10^{b}	9.64^{b}	4.32^{b}	17.98^{b}	27.36^{b}	9.48^{b}	5.82^{b}	39.38^{g}	24.82^{g}	7.49^{b}	6.97^{g}	0.16^{b}
Low	88.55^{b}	9.28^{b}	4.32^{b}	18.04^{b}	26.40^{b}	9.06^{b}	5.35^{b}	41.27^{g}	26.10^{g}	7.31^{b}	7.58^{g}	0.23^{b}
High	87.20^{b}	9.18^{c}	4.35^{b}	17.89^{b}	24.61^{c}	9.03^{c}	5.17^{c}	43.61^{h}	28.01^{h}	7.96^{b}	8.00^{h}	0.15^{b}
Sprinkling Time (T)												
1	88.29^{b}	9.25^{b}	4.31^{b}	17.93^{b}	26.24^{b}	9.12^{b}	5.63^{b}	41.10^{b}	26.26^{b}	7.36^{b}	7.44^{b}	0.14^{b}
2	87.87^{b}	9.09^{b}	4.34^{b}	18.00^{b}	25.88^{b}	8.88^{b}	5.25^{b}	41.73^{b}	26.36^{b}	7.69^{b}	7.59^{b}	0.21^{b}
Processing (P)												
Pre-baling	85.27^{g}	9.31^{b}	4.31^{b}	17.94^{b}	26.08^{b}	9.03^{b}	5.39^{b}	41.57^{b}	26.26^{b}	7.69^{b}	7.39^{b}	0.28^{e}
Pre-feeding	91.00^{h}	9.23^{b}	4.35^{b}	17.99^{b}	26.04^{b}	9.15^{b}	5.50^{b}	41.27^{b}	26.37^{b}	7.24^{b}	7.65^{b}	0.08^{f}

[a] Factors means

[b,c,d] Means of the same nutrient component and the same factor with the same superscript are not signficantly different; $P < .05$.

[e,f] Means of the same nutrient component and the same factor with the same superscript are not significantly different; $P < .01$.

[g,h] Means of the same nutrient component and the same factor with the same superscript are not significantly different; $P < .001$.

In spite of the fact that ash percent was significantly higher (P<.05) at late vegetative (Table 6), the difference between factor means was not significant (Table 7). But the decrease of ash content was reflected on soluble ash. Soluble ash averaged 9.46% vs. 8.72% at late vegetative and early bloom respectively. There was a highly significant difference (P<.001) of maturity factor effect (Table 7). Average protein percentage decreased from 18.43% to 17.5% with increasing maturity, however, there was no significant difference (Table 7). Available carbohydrates and lipids did not show any clear response to maturity. As can be seen in Table 5, low and high values are found at both stages of maturity. Cellulose, hemicellulose, and lignin as a fraction of cell walls were slightly higher in the late maturity samples (Table 7) but the differences were not statistically significant . Cell walls increased significantly (P<.05) from 40.62% to 42.21% from late vegetative to early bloom.

Artificial rain. Artificial rain proved to be a significant factor in altering the chemical composition of alfalfa hay. The content of ash, soluble ash, available carbohydrates and lipids all decreased, while cell wall constituents increased, when low and high artificial rain was applied (see Tables 4, 5 and Figure 4).

Ash in soluble ash (SA) were significantly (P<.05) reduced by the leaching effect of artificial rain applied (Table 6). Ash content decreased from 9.64% to 9.28% and 9.18% at no rain, low and high level of artificial rain applied (Table 7). SA content was reduced from 9.48% to 9.06% and 9.03% at no rain, low and high level respectively (Table 7). For both, ash and SA, a high level of artificial rain produced higher leaching effect than a low level. Acid

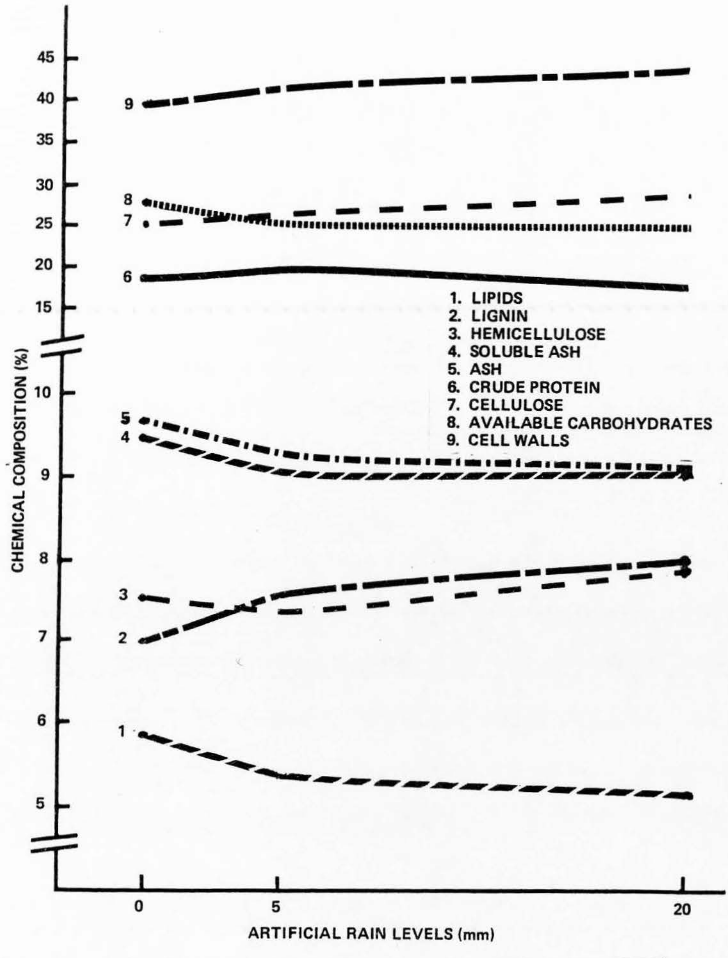


Figure 4. Effect of leaching on chemical composition of alfalfa hay. (Refer to Table 7 for data and statistical analysis).

insoluble ash (AIA) did not show any consistent effect by leaching. This effect can be associated with the results presented by Camburn (1944) because of the hay making. Shepherd et al. (1954, 1955) reported losses of ash in field cured, rained on alfalfa hay. An interesting result of the work of Shepherd et al. (1947) was that greater nutrient losses usually accompanied longer drying periods because of the rain. These results are also in agreement with Fonnesbeck et al. (1981a) showing leaching effect by rain on highly soluble ash in alfalfa hay.

Protein content was not affected significantly by artificial rain at any level. Anderson (1966), Shepherd (1959), Voelcker (1970) noted leaf, carotene and protein losses can be related to the shattering effect during drying time which became longer under the influence of rain. But, if leaves can be retained, such losses will not occur (Barnes et al., 1972; Anderson, 1966).

Available carbohydrates (AC) content of alfalfa was significantly ($P<.05$) reduced under the effect of artificial rain applied (Table 6). AC content was reduced from 27.36% to 26.40% and 24.61% at no rain, low level and high level of artificial rain applied respectively (Table 7). High level of artificial rain also produced higher leaching effect than for a low level on this fraction. Shepherd et al. (1954, 1955) reported greater losses by heavy rain on the NFE fraction of alfalfa hay which can be associated partially to the AC fraction reported in this experiment. Fonnesbeck et al. (1981a) also showed the main leaching effect by rain on available carbohydrates in alfalfa hay.

Total lipids fraction was significantly reduced (P<.05) by the leaching effect of artificial rain applied. Total lipids fraction was reduced from 5.82% at no rain to 5.17% at high level of artificial rain (Table 7). Camburn (1944) reported the reduction of ether extract fraction associated with hay making. Shepherd et al. (1954, 1955) reported losses of lipids (through the ether extract fraction) in field cured, rained on alfalfa hay.

Cellulose, hemicellulose, lignin, and the whole cell walls fraction of alfalfa increased farily consistently under the effect of artificial rain (see Tables 4 and 5). Cellulose, lignin and cell wall content increased significantly (P<.001) under the effect of artificial rain levels (Tables 6 and 7). As crude fiber and cell walls are comparable components, Carter (1960) noted that crude fiber is higher because of leaf losses. Shepherd et al. (1947, 1954, 1955) noted a lowering of the soluble and more digestible portions in alfalfa from rain damage which result in an increase in fiber content (Anderson, 1966).

Sprinkling time. Although it was expected that water would be more damaging to the dryer hay due to leaching and leaf shatter (Shepherd et al., 1947; Anderson, 1966; Tukey, 1970) no significant sprinkling time effects were observed on alfalfa chemical composition. Perhaps at the first time of sprinkling, the forage was already too dry and allowed maximum water leaching.

Process. There was no significant difference in the chemical composition of alfalfa under the effect of processing except for DM content previously discussed and acid insoluble ash content. AIA content was significantly (P<.001) affected by processing. AIA content was greater at pre-baling than for the pre-feeding samples (0.28% vs. 0.08%). AIA is not a natural component of alfalfa forage.

Maturity by artificial rain levels of interaction. A signficiant maturity by artificial rain levels interaction was observed in AC (P .05), total lipids (P .05), cellulose (P .001) and cell walls (P .05) content (Table 6). When the maturity by level of water applied for available carbohydrates (Appendix Table 16) is examined a significant decrease in AC content of alfalfa is observed due to the effect of artificial rain. AC dropped from 27.36% at no rain to 26.4% at low level and 24.61% at high level.

Maturity be level of water applied for lipids (Appendix Table 20) indicated a significant decrease from 5.82% to 5.35% and 5.17% at no water, low and high level of artificial rain applied respectively.

Cellulose percent increased significantly by maturity x level of water applied interaction (Appendix Table 24). Cellulose increased from 24.82% to 26.10% and 28.01% for no water, low and high level of water applied. There was a parallel increase in cellulose content due to stage of maturity.

Significant maturity by artificial rain interaction was observed for cell wall content Table 6). Cell wall content increased from 39.38% to 41.27% and 43.61% at no water, low and high level applied (Appendix Table 22). Cell wall content was increased by maturity effect. However, hemicellulose and lignin content were not significant for maturity by artificial rain interaction, they followed a like pattern as other fibrous constituents (Appendix Tables 26 and 28).

The effect of maturity and artificial rain has been discussed in previous sections separately.

Protein content of alfalfa hay did not show any signficiant difference in the maturity by level of water applied interaction (Appendix Table 14), but a slight change due to stage of maturity was observed as it has been indicated in a previous section.

Maturity by processing time interaction. A significant maturity by processing time interaction on gross energy ($P<.05$) and for total lipids ($P<.01$) was observed (Table 6). A slight increase of total lipids content was observed in alfalfa with increasing maturity, and also there was a slight increase due to processing time (Appendix Table 21).

Weather Observations during Harvesting

The weather was favorable for hay making during the two harvesting periods, with mostly sunny or totally clear skies, no rain, warm to very warm temperatures, and good drying conditions. The weather was considered to be about average for the area, with light wind, and low relative humidity. Drying conditions were considered very good, with warm to hot days. There was more wind during the later drying period that decreased the total drying time.

The purpose of recording weather conditions was to insure that if unusual weather occurred, that could significantly affect experimental results, measures would be available to evaluate the effects. Day and times of processing period and weather conditions are shown in Figure 5a and 5b, and on Table 8.

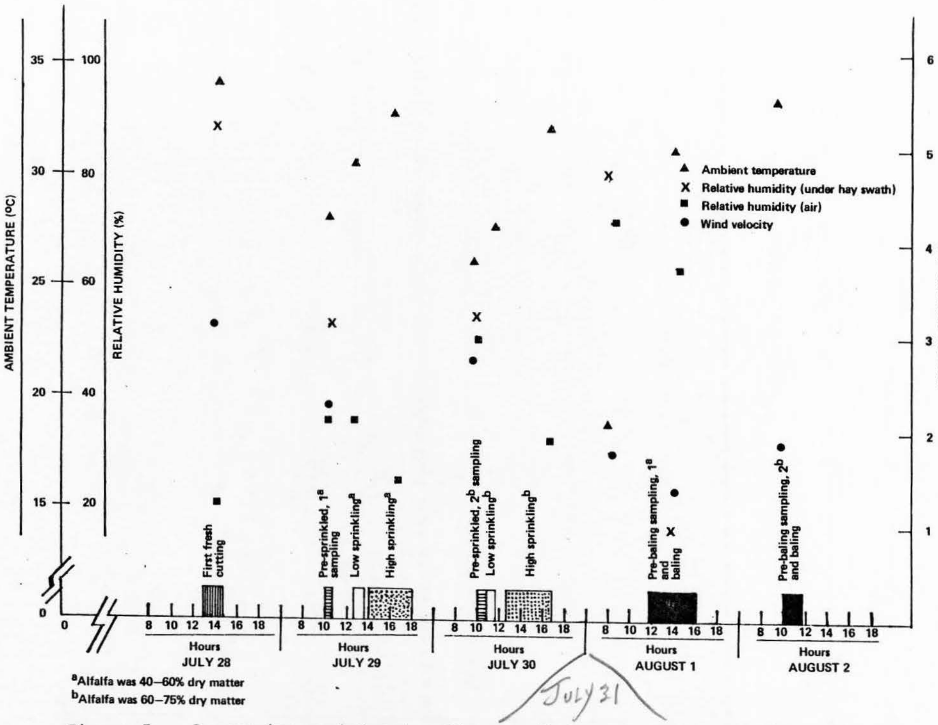
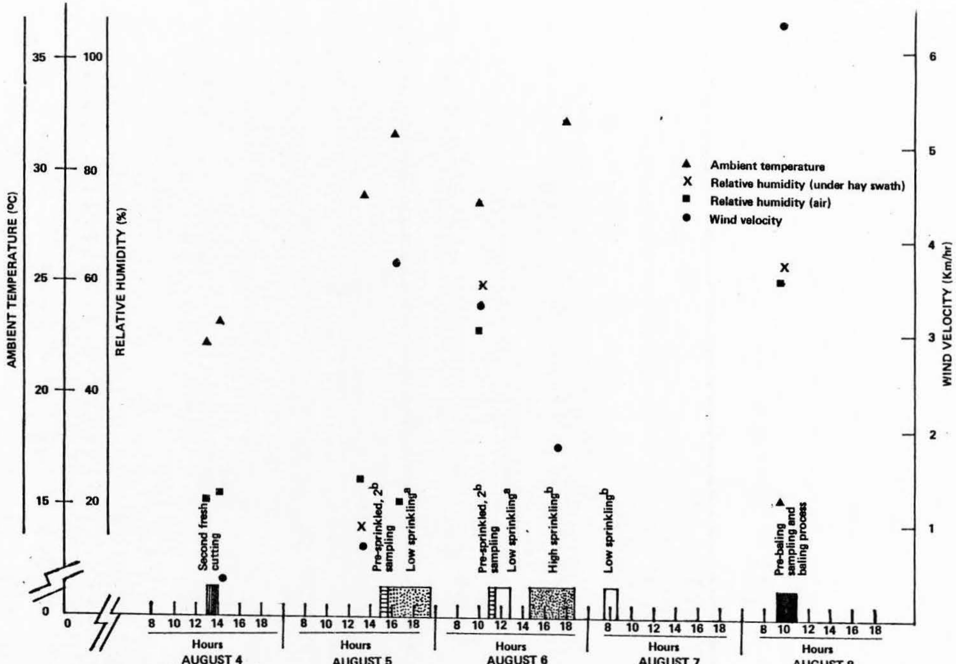


Figure 5a. Processing period and weather conditions at late vegetative.



[a]Alfalfa was 40–60% dry matter

[b]Alfalfa was 60–75% dry matter

Figure 5b. Processing period and weather conditions at early bloom.

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Table 8. Processing period and weather conditions.

Maturity	Date Days	Time Hour	Process	Ambient Temper- ature C^{o}	Relative Humidity Under Hay Swath %	Relative Humidity Air %	Wind Velocity Km/hr
Late vegetative	July 28	1:00	First fresh cut	--	--	--	--
	" "	2:00		34	89	22	3.08
	July 29	10:00	Pre-sprinkled sampling[a]	28	52	35	2.28
	" "	12:30	Low-sprinkling[a]	30.5	--	35	--
	" "	2:00	High-sprinkling[a]	--	--	--	--
	" "	4:15		32.7	--	23	--
	July 30	9:30		26	53	50	2.72
	" "	10:00	Pre-sprinkling sampling[b]	--	--	--	--
	" "	10:30	Low-sprinkling[b]	--	--	--	--
	" "	11:30		27.7	--	--	--
July 31 ?	" "	12:30	High-sprinkling[b]	--	--	--	--
	" "	4:30		32.2	--	31	--
	August 1	8:00		18.8	80	71	1.74
	" "	11:30	Pre-baling sampling and baling	--	--	--	--
	" "	2:00		31	--	--	1.34
	August 2	9:40		33	--	--	1.84
	" "	10:00	Pre-baling sampling and baling	--	--	--	--
Early bloom	August 4	1:00	Second fresh cutting	22	--	20	--
	" "	2:00		23	--	21	.34
	August 5	1:00		28.8	16	24	.67
	" "	3:00	Pre-sprinkled sampling[a]	--	--	--	--
	" "	4:00	High-sprinkling[a]	31.6	20	20	3.69

Table 8. (Continued)

Maturity	Date Days	Time Hour	Process	Ambient Temper-ature C°	Relative Humidity Under Hay Swath %	Relative Humidity Air %	Wind Velocity Km/hr
Early bloom	August 6	10:00		28.8	59	51	3.21
	" "	11:00	Pre-sprinkled sampling[b]	--	--	--	--
	" "	11:30	Low-sprinkling[a]	--	--	--	--
	" "	2:30	High sprinkling[b]	--	--	--	--
	" "	5:00		32.2	--	--	1.07
	August 7	7:30	Low sprinkling[b]	--	--	--	--
	August 8	9:30	Pre-baling sampling and baling	20	62	60	6.14

[a]Sprinkling time when alfalfa was about 40 to 60% dry matter.

[b]Sprinkling time when alfalfa was about 60 to 75% dry matter.

Results of Grading Alfalfa Hay

Quality of alfalfa hay was estimated through the new hay-grading system of Fonnesbeck and Anderson (1981). Based on chemical composition of the hay the relative value index (RVI) of the alfalfa hay was estimated for a specific combination of crude protein (CP) and cell wall (CW) content. CW is about 3% less than neutral detergent fiber (NDF) for alfalfa hay (Fonnesbeck et al., 1981). Therefore, a table was generated by replacing NDF with a CW value 3% less than the NDF value (see Table 9). The RVI value as it was obtained by the intersection of the CP line by the CW column in Table 9 is shown on Tables 4 and 5. According to this method, the RVI can be used to trade hay at a price favorable to the buyer and seller, because generally there is no central hay marketing organization to regulate the price based on quality and demand for hay in a given area. The comparative effect of CP and CW interaction of alfalfa by maturity, levels of artificial rain, and treatment means are shown in Figures 6, 7 and 8 respectively.

Table 9. Relative value of alfalfa hay estimated from crude protein and CW content (dry basis).

Crude Protein %	Cell Wall, %										
	32	34	36	38	40	42	44	46	48	50	52
					Relative value index						
24	1.161	1.142	1.122	1.103	1.083	1.064	---	---	---	---	---
23	1.147	1.128	1.108	1.089	1.069	1.050	1.031	---	---	---	---
22	1.133	1.114	1.094	1.075	1.056	1.036	1.017	.997	---	---	---
21	1.119	1.020	1.080	1.061	1.042	1.022	1.003	.983	.964	---	---
20	1.105	1.086	1.067	1.047	1.028	1.008	.989	.970	.950	.931	---
19	1.092	1.072	1.053	1.033	1.014	.995	.975	.956	.936	.917	.898
18	1.078	1.058	1.039	1.019	1.000	.981	.961	.942	.922	.903	.884
17	---	1.044	1.025	1.006	.986	.967	.947	.928	.908	.889	.870
16	---	---	1.011	.992	.972	.953	.933	.914	.895	.875	.856
15	---	---	---	.978	.958	.939	.920	.900	.881	.861	.842
14	---	---	---	---	.944	.925	.906	.886	.867	.847	.828
13	---	---	---	---	---	.911	.892	.872	.853	.834	.814
12	---	---	---	---	---	---	.878	.858	.839	.820	.800

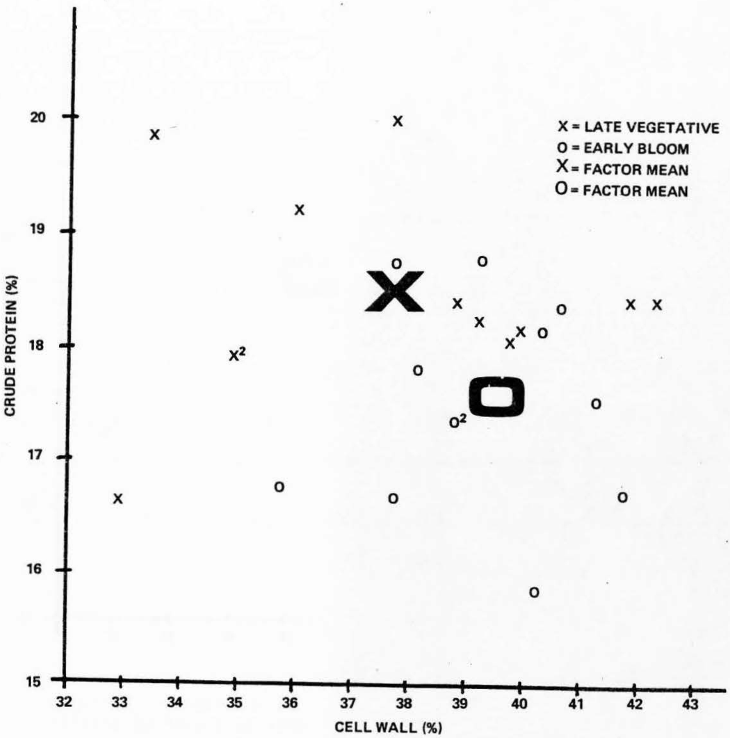

Figure 6. Comparison of crude protein and cell wall content of alfalfa by stage of maturity.

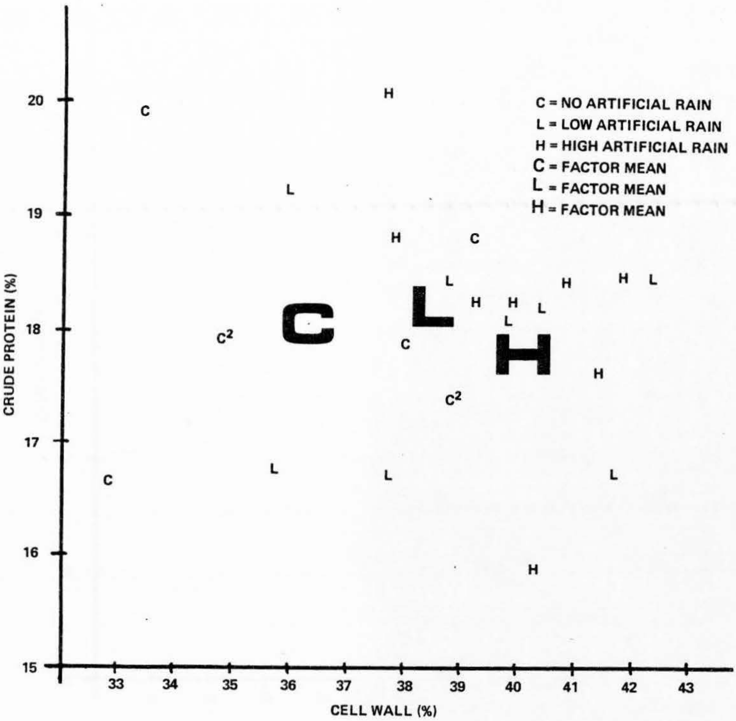

Figure 7. Comparison of crude protein and cell wall content of alfalfa by levels of artificial rain.

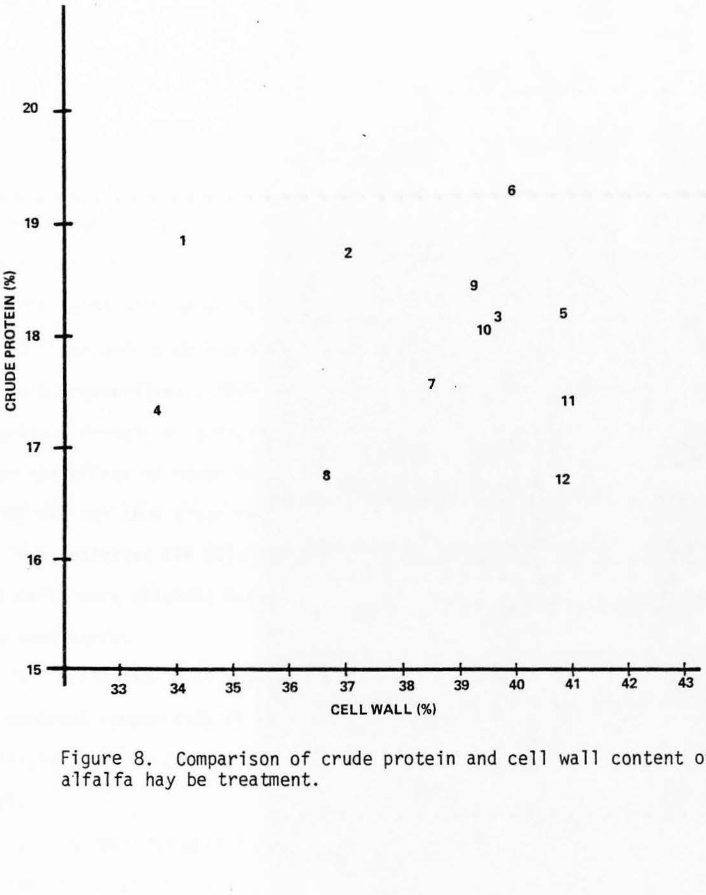

Figure 8. Comparison of crude protein and cell wall content of alfalfa hay be treatment.

SUMMARY AND CONCLUSIONS

1. Twelve treatments of alfalfa hay in a factorial experimental design were collected to obtain the chemical composition attributes as influenced by stage of maturity, artificial rain, sprinkling time and processing.

2. With advancing maturity, the alfalfa samples showed significantly lower plant cell content percentages, while cell wall constituents increased. Dry matter yield increased from 2,724 Kg/ha to 3,206 Kg/ha with advancing maturity.

3. Ash and soluble ash decreased significantly ($P<.05$) and ($P<.001$), respectively, from late vegetative to early bloom stage of maturity. Protein as a fraction of the cell content was also lowered under the effect of stage of growth, but available carbohydrates and lipids did not show clear response to this factor.

4. Cellulose ($P<.05$), hemicellulose and lignin as a fraction of cell walls were slightly higher in the early bloom maturity than at late vegetative.

5. Artificial rain proved to be a significant factor in altering chemical composition of alfalfa hay by leaching. High level of artificial rain produced higher leaching effect than for the low level.

a. The most soluble fraction of the plant cell content suffered greater effect of leaching by artificial rain. The more soluble minerals were leached significantly and reflected in the reduction ($P<.05$) of ash and soluble ash fraction of alfalfa. Also, highly readily available carbohydrates fraction was lowered significantly (P .05) under the leaching effect of water.

b. Total lipids was slightly reduced (P .05) by the leaching effect, but protein content was not affected.

c. Percentage of cell wall constituents increased significantly (P .001) by rain damage because of the increase in fiber content and lowering of the soluble portions in alfalfa for leaching.

d. A significant maturity by artificial rain levels interaction was observed in available carbohydrates, total lipids, cellulose and plant cell wall fraction. While available carbohydrates and total lipids were reduced, cellulose and total cell wall fraction increased.

6. The difference among sampling areas in the experimental plot showed that there is a definite need to carry out experiments such as this on more experimental units for replication of treatments and large samples. In future experiments, it will be necessary to reduce the effect of field variation in quantity and quality of alfalfa hay with more replications. For example, we could not estimate yield losses from this field as was planned in one of the main objectives of this experiment because of the variability within the experimental plot. Perhaps having a more controllable area to be harvested and greater land area, the land could be planted to forage crops for future research in this area.

7. It will be helpful in future research in this area to notice that sprinkling time and processing factors showed no interaction. It may not be necessary to study those factors in combinations.

8. It is suggested that if in the future planning experiments of this kind can have cooperation from the University Experimental Station farm, will be beneficial and very helpful.

9. Hay dried under ideal conditions so effect of rain was predominantly from leaching rather than respiration losses.

10. This was an initial experiment in a new area of research. The results and observations reported here should contribute to the improvement of experimental techniques and our knowledge in this area.

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APPENDIX

Table 10. Average dry matter percentage of alfalfa hay harvested at different stages of maturity and with different levels of water applied.

	Level of Water Applied			
Stage of Maturity	C	L	H	Average
Late vegetative	87.42[a] (4)	89.19 (4)	88.48 (4)	88.37 (12)
Early bloom	88.77 (4)	88.71 (4)	85.91 (4)	87.80 (12)
Average	88.10 (8)	88.95 (8)	87.20 (8)	

[a]Number of observations are in parenthesis.

Table 11. Average dry matter percentage of alfalfa hay harvested at different stages of maturity and at pre-baling or pre-feeding.

	Processing time		
Stage of Maturity	Pre-baling	Pre-feeding	Average
Late vegetative	86.11[a] (6)	90.62 (6)	88.37 (12)
Early bloom	84.24 (6)	91.35 (6)	87.80 (12)
Average	85.27 (12)	91.00 (12)	

[a]Number of observations are in parenthesis.

Table 12. Average ash percentage of alfalfa hay harvested at different stages of maturity and with different levels of water applied.

	Level of Water Applied			
Stage of Maturity	C	L	H	Average
Late vegetative	9,97[a] (4)	9.46 (4)	9.44 (4)	9.62 (12)
Early bloom	9.31 (4)	8.51 (4)	8.94 (4)	8.92 (12)
Average	9.64 (8)	8.99 (8)	9.18 (8)	

[a]Number of observations are in parenthesis.

Table 13. Average ash percentage of alfalfa hay harvested at different stages of maturity and at pre-baling or pre-feeding.

	Processing Time		
Stage of Maturity	Pre-baling	Pre-feeding	Average
Late vegetative	9.67[a] (6)	9.57 (6)	9.62 (12)
Early bloom	8.94 (6)	8.89 (6)	8.92 (12)
Average	9.31 (12)	9.23 (12)	

[a]Number of observations are in parenthesis

Table 14. Average protein percentage of alfalfa hay harvested at different stages of maturity and with different levels of water applied.

	Level of water applied			
Stage of Maturity	C	L	H	Average
Late vegetative	18.09[a] (4)	18.49 (4)	18.72 (4)	18.43 (12)
Early bloom	17.86 (4)	17.59 (4)	17.05 (4)	17.50 (12)
Average	17.98 (8)	18.04 (8)	17.89 (8)	

[a]Number of observations are in parenthesis.

Table 15. Average protein percentage of alfalfa hay harvested at different stages of maturity and at pre-baling or pre-feeding.

	Processing Time		
Stage of Maturity	Pre-baling	Pre-feeding	Average
Late vegetative	18.26[a] (6)	18.60 (6)	18.43 (12)
Early bloom	17.62 (6)	17.38 (6)	17.50 (12)
Average	17.94 (12)	17.99 (12)	

[a]Number of observations are in parenthesis.

Table 16. Average available carbohydrates percentage of alfalfa hay harvested at different stages of maturity and with different levels of water applied.

	Level of Water Applied			
Stage of Maturity	C	L	H	Average
Late vegetative	28.95[a] (4)	25.33 (4)	23.99 (4)	26.09 (12)
Early bloom	25.77 (4)	27.08 (4)	25.23 (4)	26.02 (12)
Average	27.36 (8)	26.46 (8)	24.61 (8)	

[a]Number of observations are in parenthesis.

Table 17. Average available carbohydrates percentage of alfalfa hay harvested at different stages of maturity and at pre-baling or pre-feeding.

	Processing Time		
Stage of Maturity	Pre-baling	Pre-feeding	Average
Late vegetative	26.74[a] (6)	25.45 (6)	26.10 (12)
Early bloom	25.41 (6)	26.63 (6)	26.02 (12)
Average	26.08 (12)	26.04 (12)	

[a]Number of observations are in parenthesis.

Table 18. Average soluble ash percentage of alfalfa hay harvested at different stages of maturity and with different levels of water applied.

	Level of Water Applied			
Stage of Maturity	C	L	H	Average
Late vegetative	9.83[a] (4)	9.43 (4)	9.11 (4)	9.46 (12)
Early bloom	9.13 (4)	8.68 (4)	8.36 (4)	8.72 (12)
Average	9.48 (8)	9.06 (8)	8.74 (8)	

[a]Number of observations are in parenthesis.

Table 19. Average soluble ash percentage of alfalfa hay harvested at different stages of maturity and at pre-baling or pre-feeding.

	Processing Time		
Stage of Maturity	Pre-baling	Pre-feeding	Average
Late vegetative	9.37[a] (6)	9.54 (6)	9.46 (12)
Early bloom	8.69 (6)	8.75 (6)	8.72 (12)
Average	9.03 (12)	9.15 (12)	

[a]Number of observations are in parenthesis.

Table 20. Average of total lipids percentage of alfalfa hay harvested at different stages of maturity and with different levels of water applied.

	Level of Water Applied			
Stage of Maturity	C	L	H	Average
Late vegetative	6.15[a] (4)	5.02 (4)	4.84 (4)	5.34 (12)
Early bloom	5.48 (4)	5.67 (4)	5.50 (4)	5.55 (12)
Average	5.82 (8)	5.35 (8)	5.17 (8)	

[a]Number of observations are in parenthesis.

Table 21. Average total lipids percentage of alfalfa hay harvested at different stages of maturity and with different levels of water applied.

	Processing Time		
Stage of Maturity	Pre-baling	Pre-feeding	Average
Late vegetative	4.97[a] (6)	5.70 (6)	5.34 (12)
Early bloom	5.81 (6)	5.29 (6)	5.55 (12)
Average	5.39 (12)	5.50 (12)	

[a]Number of observations are in parenthesis.

Table 22. Average plant cell walls percentage of alfalfa hay harvested at different stages of maturity and with different levels of water applied.

	Level of Water Applied			
Stage of Maturity	C	L	H	Average
Late vegetative	36.99[a] (4)	41.54 (4)	43.35 (4)	40.63 (12)
Early bloom	41.77 (4)	41.10 (4)	43.87 (4)	42.21 (12)
Average	39.38 (8)	41.27 (8)	43.61 (8)	

[a]Number of observations are in parenthesis.

Table 23. Average plant cell walls percentage of alfalfa hay harvested at different stages of maturity and at pre-baling or pre-feeding.

	Processing Time		
Stage of Maturity	Pre-baling	Pre-feeding	Average
Late vegetative	40.66[a] (6)	40.58 (6)	40.62 (12)
Early bloom	42.47 (6)	41.95 (6)	42.21 (12)
Average	41.67 (12)	41.27 (12)	

[a]Number of observations are in parenthesis.

Table 24. Average cellulose percentage of alfalfa hay harvested at different stages of maturity and with different levels of water applied.

	Level of Water Applied			
Stage of Maturity	C	L	H	Average
Late vegetative	23.15[a] (4)	26.62 (4)	27.87 (4)	25.88 (12)
Early bloom	26.49 (4)	25.58 (4)	28.15 (4)	26.74 (12)
Average	24.82 (8)	26.10 (8)	28.01 (8)	

[a]Number of observations are in parenthesis.

Table 25. Average cellulose percentage of alfalfa hay harvested at different stages of maturity and at pre-baling or pre-feeding.

	Processing Time		
Stage of Maturity	Pre-baling	Pre-feeding	Average
Late vegetative	25.81[a] (6)	25.95 (6)	25.88 (12)
Early bloom	26.70 (6)	26.78 (6)	26.74 (12)
Average	26.26 (12)	26.37 (12)	

[a]Number of observations are in parenthesis.

Table 26. Average hemicellulose percentage of alfalfa hay harvested at different stages of maturity and with different levels of water applied.

	Level of Water Applied			
Stage of Maturity	C	L	H	Average
Late vegetative	7.34[a] (4)	7.11 (4)	7.12 (4)	7.19 (12)
Early bloom	7.63 (4)	7.50 (4)	8.80 (4)	7.98 (12)
Average	7.49 (8)	7.31 (8)	7.96 (8)	

[a]Number of observations are in parenthesis.

Table 27. Average hemicellulose percentage of alfalfa hay harvested at different stages of maturity and at pre-baling or pre-feeding.

	Processing Time		
Stage of Maturity	Pre-baling	Pre-feeding	Average
Late vegetative	7.18[a] (6)	7.19 (6)	7.19 (12)
Early bloom	8.19 (6)	7.28 (6)	7.74 (12)
Average	7.69 (12)	7.24 (12)	

[a]Number of observations are in parenthesis.

Table 28. Average lignin percentage of alfalfa hay harvested at different stages of maturity and with different levels of water applied.

	Level of Water Applied			
Stage of Maturity	C	L	H	Average
Late vegetative	6.62[a] (4)	7.68 (4)	7.81 (4)	7.37 (12)
Early bloom	7.31 (4)	7.48 (4)	8.19 (4)	7.66 (12)
Average	6.97 (8)	7.58 (8)	8.0 (8)	

[a]Number of observations are in parenthesis.

Table 29. Average lignin percentage of alfalfa hay harvested at different stages of maturity and at pre-baling or pre-feeding.

	Processing Time		
Stage of Maturity	Pre-baling	Pre-feeding	Average
Late vegetative	7.22[a] (6)	7.52 (6)	7.37 (12)
Early bloom	7.55 (6)	7.77 (6)	7.66 (12)
Average	7.39 (12)	7.65 (12)	

[a]Number of observations are in parenthesis.

VITA

Mercedes M. García de Hernández

Candidate for the Degree of

Master of Science

Report: Effect of Rain Leaching on Chemical Composition of Alfalfa Hay

Major Field: Animal Nutrition

Biographical Information:

Personal Data: Born at Acarigua, Portuguesa, Venezuela June 9, 1953; married Emiliano Hernandez, December 29, 1979.

Education: Received the Bachelor Degree of Veterinary Physician at University of Zulia in 1976. Completed requirements for the Master of Science degree in Animal Nutrition at Utah State University in 1981.

Work Experience: Research Assistant at Fondo Nacional de Investigaciones Agropecuarias, El Cuji Experimental Station, Barquisimeto, Venezuela from 1976 to present.