FORAGE CONSTITUENTS

Diurnal Variation in Composition of Alfalfa

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The diurnal changes in composition of alfalfa tops taken from four plots of prebloom Ranger alfalfa at four different times in 1957 and 1958 are reported. Significant ($P < 0.01$) diurnal changes in dry matter, total nitrogen, reducing sugars, and phosphorus contents were observed. Changes in ash content were significant at $P = 0.05$. Diurnal changes in nonprotein nitrogen, amino nitrogen, and calcium contents were not significant. There were significant among-plot differences for all constituents analyzed. Time of day had no appreciable effect on levels of citric, malic, malonic, and total nonvolatile organic acids. There was no substantial evidence to indicate that diurnal changes in components measured were closely related to the incidence of bloat.

INFORMATION on daily variations in the composition of alfalfa is limited. In addition to the obvious relationship to nutritional value, such changes might possibly be related to certain animal disorders. Bloat occurs with greater frequency in late afternoon and early morning. Although grazing habits and other factors may be involved in bloat incidence, it seemed desirable to ascertain the nature of diurnal changes, which in turn might be related to animal response. The objective of the present study was to determine diurnal changes in several components of alfalfa and variations within plots and among noncontemporary plots. In order to simulate the type of forage consumed by the grazing animal, this study was restricted to analysis of the top portion of plants.

Experimental

A plot of prebloom Ranger alfalfa was selected at each of the following times: May 22, 1957; May 14, 1958; August 29, 1958; and September 11, 1958. The first three were selected, one each, from three adjacent fields, while the September 1958 plot was in the same general area as the August 1958 plot. All plots were on Color series, clay loam soil at Ames, Iowa. Each plot was divided into three subplots and a forage sample was taken from each subplot at 6 A.M., 10 A.M., 2 P.M., 6 P.M., and 10 P.M. (C.S.T.). A stratified random sampling plan within subplots was employed to obtain each sample. Six sets of 25 alfalfa tops (approximately 4 inches in length), each set from a different area in the subplot, were obtained and pooled to form a sample. During the initial grazing of alfalfa pasture, cattle tend to select the tops rather than consuming most of the above-ground portion of the plant. Therefore, in this study the tops were selected as being most representative of the material consumed when bloat is likely to be most intense.

Each sample was placed in a plastic bag and quickly frozen in a chest which contained dry ice. Subsequently, the samples were stored in a frozen state until ready for chemical analysis. Then each sample was chopped, while still frozen, and aliquots were taken for analysis for dry matter, ash, total nitrogen, nonprotein nitrogen, amino acid nitrogen, calcium, phosphorus, and reducing sugar. For total nitrogen, a micro adaptation of the method of Perrin (*11*) on dry, ground material was employed. Nonprotein nitrogen was determined by the micro-Kjeldahl technique (*11*) on a protein-free filtrate prepared by mixing, in a Waring Blendor, 8 to 10 grams of sample with 100 ml. of a solution containing 1% of trichloroacetic acid and 5% of NaCl plus 2 grams of activated charcoal as a decolorizing agent. Amino acid nitrogen was determined by a modification of the 1,2-naphthoquinone-4-sulfonic acid method (*6*) on the nonprotein nitrogen filtrate prepared above. Calcium was determined by the method of Weybrew, Matrone, and Baxley (*14*), phosphorus by the technique of Chen, Toribara, and Warner (*3*), and reducing sugar by a slight modification of the method of Wiseman, Mallack, and Jacobson (*15*). Samples from the May 1957 plot were analyzed for organic acids (total, citric, malonic, and malic). The total organic acids were determined by extracting samples with 80% ethyl alcohol in a Waring Blendor, filtering, washing with 80% ethyl alcohol until no green color remained on the residue, passing the combined filtrate and washings through a Dowex 1-X8 column, washing the column with 80% ethyl alcohol until the effluent liquid was colorless, washing with water to remove the alcohol, and then proceeding with collection and determination of total organic acids by the method of Resnik, Lee, and Powell (*12*). Individual acids were separated by ascending chromatography on Whatman 3 MM paper, using a butyl alcohol–formic acid–water system (*9*), and spraying with a 0.04% solution of Bromophenol Blue adjusted to pH 7 with sodium hydroxide. Quantitative estimation of various acids was made using a spot weight essentially the same as that described by Bryant and Overell (*2*).

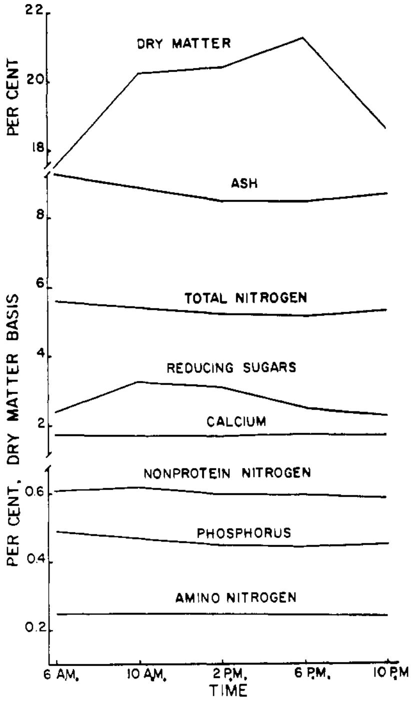

Figure 1. Diurnal variation in certain components of alfalfa tops

Data obtained were subjected to analysis of variance and to Duncan's multiple range test (*5*).

Results

The effect of time of day on mean values for various alfalfa components is shown in Figure 1. Each value at each of the six sampling times is the mean for 12 samples, three from each of the four

Table I. Summary of Analysis of Variance of Alfalfa Components

Source of Variation	df	F Values							
		DM[a]	Total N	Nonprotein N	Amino N	Reducing sugars	Ash	Ca	P
Plots	3	21.25^b	82.57^b	335.80^b	115.72^b	19.52^b	8.71^b	29.01^b	356.44^b
Subplots: plots	8	1.57	3.60^b	1.10	1.40	0.51	1.09	1.21	0.85
Hours	4	7.23^b	7.58^b	2.00	0.62	8.89^b	3.41^c	0.55	12.67^b
Plots × hours	12	12.21^b	5.22^b	0.90	1.14	5.50^b	3.70^b	1.50	1.57
Hours × subplots: plots	32								

[a] DM = dry matter. [b] $P < 0.01$. [c] $P < 0.05$.

Table II. Mean Values for Various Alfalfa Components for Four Plots

(15 samples per plot)

Plot[a]	DM,[b] %	Total N	Nonprotein N	Amino N	Reducing Sugars	Ash	Ca	P
		DM Basis, %						
May 1957	20.85	5.19	0.585	0.212	2.73	8.55	1.82	0.478
May 1958	20.37	4.83	0.454	0.187	3.45	8.68	1.88	0.390
Aug. 1958	16.13	6.24	0.807	0.330	2.20	9.54	1.55	0.570
Sept. 1958	21.17	5.11	0.548	0.250	2.50	8.39	1.49	0.402

[a] In each plot, three subplots were sampled at five times (6 and 10 A.M.; 2, 6, and 10 P.M.). [b] DM = dry matter.

Table III. Duncan's Multiple Range Test on Those Alfalfa Components Which Displayed Significant Diurnal Variations

Component	Sample Times, Mean Values[a]				
Total nitrogen	6 P.M. 5.17	2 P.M. 5.21	10 P.M. 5.34	10 A.M. 5.40	6 A.M. 5.60
Reducing sugars	10 P.M. 2.33	6 A.M. 2.38	6 P.M. 2.56	2 P.M. 3.10	10 A.M. 3.24
Phosphorus	6 P.M. 0.443	2 P.M. 0.446	10 P.M. 0.454	10 A.M. 0.469	6 A.M. 0.487
Ash	6 P.M. 8.50	2 P.M. 8.51	10 P.M. 8.69	10 A.M. 8.89	6 A.M. 9.36
Dry matter, %	6 A.M. 17.57	10 P.M. 18.64	10 A.M. 20.23	2 P.M. 20.41	6 P.M. 21.31

[a] Except for dry matter, all values are expressed on a dry matter basis.

plots. Samples from the August 1958 plot were taken when the alfalfa was about 8 to 10 inches high and was very lush; consequently, the mean levels of total nitrogen, nonprotein nitrogen, amino nitrogen, and phosphorus were substantially higher and those for dry matter, reducing sugar, and calcium were lower in samples from this plot than values for samples from the other plots. Since diurnal trends were similar to those of the other plots, data for August 1958 samples are included with the data of Figure 1. Components which changed most during the day were reducing sugars and dry matter. There was a marked increase in the level of reducing sugar between 6 and 10 A.M., followed by a gradual decline at 10 P.M. Dry matter content increased to a maximum at 6 P.M., then declined sharply. Ash, phosphorus, and total nitrogen declined gradually to minima at either 2 or 6 P.M., then increased slightly. Values for calcium, nonprotein nitrogen, and amino nitrogen did not follow any specific diurnal pattern and changes were small.

All data shown in Figure 1 were subjected to analysis of variance and resulting F values are summarized in Table I. All sources of variation were considered random variates and appropriate error terms were used.

There were significant ($P < 0.01$) differences between plots in values for all components. Exclusion of the August 1958 plot (young, lush forage) sharply reduced the F values for plots in each of these components; however, significant plot differences still existed in nonprotein nitrogen, amino nitrogen, calcium, and phosphorus values. The extent of variation between mean values for each plot is shown in Table II. Values for the August plot are either highest or lowest (except for calcium) for each component, an indication of young, rapidly growing plant tissue.

The only component which showed a significant ($P < 0.01$) subplot:plot difference was total nitrogen. It is possible, however, that slight field or laboratory sampling error could account for the differences observed.

There were significant ($P < 0.01$) diurnal changes in dry matter, total nitrogen, reducing sugars, and phosphorus. Changes in ash values were significant at $P = 0.05$. Excluding the August 1958 plot from the combined analysis did not change any conclusions on diurnal variations drawn from the analysis of all four plots. These components were subjected to Duncan's multiple range test and results are summarized in Table III. Values which are underlined do not differ significantly from one another.

Total nitrogen and phosphorus contents at 6 A.M. were significantly higher than at any other time of the day. Reducing sugar contents were higher at 10 A.M. and 2 P.M., but there was no real difference between values at these two times. Ash content was highest at 6 A.M., but was not significantly higher than that at 10 A.M. Dry matter contents in samples obtained at 6 P.M. were significantly higher than those taken at 6 A.M. or 10 P.M.

There were significant ($P < 0.01$) plot × hours interactions with dry matter, total nitrogen, reducing sugars, and ash values.

The effect of time of day on levels of citric, malic, and malonic acids, and on total nonvolatile organic acids for the May 1957 series was determined. Analysis of variance of these data indicated that there were no real diurnal variations in any of the acids and also that there were no significant differences among subplots. Mean values (millequivalents per gram of dry matter) for citric, malonic, malic, and total nonvolatile organic acids, respectively, were 0.03, 0.10, 0.16, and 0.49.

Other acids which were identified, but not present in amounts sufficient for satisfactory quantitative estimation by the methods employed, included succinic, fumaric, and glycolic acids. Traces of quinic, shikimic, and phosphoric acids were found in some samples.

Discussion

These data demonstrate significant diurnal changes in composition of alfalfa tops. No data could be found in the literature regarding the composition of alfalfa tops, as collected in this experiment, but limited data are available regarding changes in composition of the alfalfa plant (above ground portion) at various times during the day. Curtis (*4*) reported that alfalfa samples cut early in the morning were lower in carbohydrate content than samples cut in the afternoon, and that total dry matter per unit area of field also increased in the afternoon. Later work by Woodward, Shepherd, and Tysdal (*16*) failed to confirm these observations, but did show a decline in ash content from 7 A.M. to 5 P.M., followed by a rise to maximum ash content at 3 A.M. Present data tend to confirm the general pattern in diurnal change in ash content of the data of Woodward, Shepherd, and Tysdal (*16*), although the value of alfalfa tops was approximately 2 to 2.5% higher than that for the whole plant (*16*), on a dry matter basis. Woodward and coworkers report no change in total nitrogen content in alfalfa through the 24-hour period. In data reported herein, the total nitrogen content of tops declined significantly from 6 A.M. until 6 P.M. and then increased slightly. Jones, Zscheile, and Griffith (*8*) observed that variations in carotene and protein contents in alfalfa occurred during the day and from day to day; however, data were not sufficient to establish the nature of diurnal trends.

Differences in composition between the alfalfa as cut for hay and in the alfalfa tops as sampled in the present study may be attributable, at least in part, to the differences in portion of the plant sampled for analysis. Also, in the present work, all samples were taken from prebloom alfalfa, whereas those reported by Woodward and associates varied in stage of growth from 18% bloom for Buffalo alfalfa to 42% bloom for Grimm alfalfa.

There were appreciable variations among plots in the level of certain components in alfalfa tops, probably due at least in part to stage of maturity. Although all of the samples analyzed in this study were taken from prebloom alfalfa, the height of the above-ground portion of the plants varied from approximately 8 inches (August 1958 plot) to approximately 16 inches (May 1957 plot). In young, rapidly growing alfalfa (illustrated by the August 1958 plot, Table II), nitrogen components were markedly higher than in samples of tops from the other plots which were more mature. Samples taken from alfalfa which was 2 weeks more mature (September 11, 1958) contained lower levels of all of the nitrogen components analyzed in this study. The ratio of nonprotein to total nitrogen was highest (0.129) for the August 1958 samples and lowest (0.094) for the May 1958 samples. The calcium to phosphorus ratio was lowest for the August 1958 samples.

When samples were taken, weather conditions were somewhat variable. The sky was clear to slightly overcast at the beginning of each sampling day. Light rains fell just prior to samples taken at 10 P.M. on May 22, 1957; at 2 P.M. on May 14, 1958; and at 10 P.M. on August 29, 1958. There was a heavy dew on the forage at 10 P.M. on May 22, 1957; August 29, 1958; and September 11, 1958. Thus, surface moisture influenced the dry matter content of several samples. Consequently, all of the data reported in this study are based upon dry matter composition.

A plots × hours interaction is caused by the failure of differences between hours to be the same from plot to plot. In the case of dry matter, this interaction was caused, at least in part, by rain at different times of the day for the four plots. For the other components (total nitrogen, reducing sugar, and ash) showing large plots × hours interactions, the explanation of this interaction is not apparent.

In an effort to obtain more information on the effect of rainfall on composition of alfalfa, effects of irrigation were evaluated in another study at Iowa State University (*1*). Four-inch tops of prebloom alfalfa were taken by a stratified random sampling procedure from two nonirrigated plots and from plots on which irrigation (sprinkling with 5.5 inches of water over a 7- to 8-hour period) had been completed approximately 0.5, 9, 18, 27, and 37 hours before sampling. All plots were adjacent areas of one field. Irrigation had little effect on reducing sugar, total nitrogen, amino nitrogen, ash, and phosphorus values. Nonprotein contents (on a dry matter basis) for various plots were: nonirrigated, 0.59%; 0.5 hour, 0.54%; 9 hours, 0.59%; 18 hours, 0.67%; 27 hours, 0.75%; and 37 hours, 0.70%. Subsequent observations indicated that calcium levels remained relatively constant. Potassium values 0.5 hour after irrigation were substantially higher than those of tops from nonirrigated plots and declined to minimum levels at 27 hours.

Rosen, Fassel, and Nichols (*13*) and Nichols and Penn (*10*) reported that certain nonvolatile organic acids of legumes are capable of increasing frothing of rumen digesta. However, in the present study there was no apparent relationship between levels of any of the nonvolatile organic acids and the bloating potential of alfalfa.

Although this study demonstrates that certain chemical constituents in alfalfa tops vary in content during the day, there is no substantial evidence that any of the changes in these constituents is directly related to the development of the bloat syndrome. In studies at this station, cattle grazing alfalfa for a 3-hour period in the morning (6:30 to 9:30 A.M.) and a 3-hour period in the afternoon (4 to 7 P.M.) bloated more during and subsequent to the late afternoon grazing period, although under some circumstances bloat has been observed to be more severe in the morning (*7*). Data presented in Figure 1 show that ash, total nitrogen, and total phosphorus were at minimum levels at 6 P.M., but it seems unlikely that the slight differences in content observed between morning samples and afternoon samples could account for a difference in bloat pattern observable in cattle grazing alfalfa.

Studies on composition of alfalfa are being continued in an effort to determine which components are related to incidence and severity of bloat in cattle and sheep.

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