Journal of Applied Biological Sciences 9 (2): 68-76, 2015
ISSN: 1307-1130, E-ISSN: 2146-0108

Determining of Some Cell Wall Compenents on Alfalfa's Cultivars in Central Anatolian Conditions

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Received: July 05, 2015
Accepted: August 17, 2015

Abstract

Alfalfa (*Medicago sativa* L.) maturity at the time of harvest greatly influences forage quality. The main objective of this research was investigation on effects of phenological stages (five diffrent stage times) on values of forage quality indices of Alfalfa. Bilensoy, Kayseri, Gozlu and Plato cultivars (dormant), Elçi, Mırna, MA-414 and Posovina (non-dormant) cultivars of Alfalfa were used as materials. Samples were collected from Reseach Field of Ankara University in Ankara. They were dried, grained and analyzed in Laboratory. The results showed that forage quality indices values including forage acid detergent fiber (ADF), neutral detergent fiber (NDF) and total digestible nutrients (TDN), dry matter intake (DMI), digestible dry matter (DDM) and relative feed value (RFV) were significantly differed culture variety and five different stage times P<0.01). For all culture variety DMI, DDM, TDN decreased and ADF, NDF, increased with plant growth development. Considering forage quality indices values among tree culture variety, Bilensoy had highest forage quality. Among life forms, forbs, higher forage quality obtained from forbs. In terms of growth stage, vegetative growth stage had better forage quality.

Keywords: Forage quality, growth stage, crude protein, acid detergent fiber, dry matter digestibility.

INTRODUCTION

Alfalfa, the queen of forages, is the main legume used for livestock feed in Turkey. Alfalfa is one of the most commonly used legumes for both hay and pasture in Turkey because of its high yield, high nutritional quality, ability to fix nitrogen, and vigorous fall regrowth [1].

Forage quality, and therefore ruminal degradability, is influenced by several factors, with the most important stage of maturity of forage, forage species, environmental effects (location in combination with temperatures and precipitation), agronomic management, site of growth, and processing such as treatment and preservation [2, 3, 4]. Alfalfa maturity plays a large role in the quality of harvested forage. The inverse relationship of advancing alfalfa maturity and declining forage quality is well established [5, 6, 7].

Fall Dormancy (FD) of the variety is an important predictor of quality. More dormant varieties were almost always higher in quality. Cutting frequency (CF), phenological stages of alfalfa is a critical factor influencing both productivity and persistence [8], but there is a lack of knowledge of how alfalfa cultivars with contrasting fall dormancy (FD) respond to CF. FD is defined as the reduction in shoot growth in the autumn due to decreasing temperatures and day length [9] and it is a useful trait that defines alfalfa adaptation to different regions. Dormant cultivars have reduced shoot elongation and decumbent shoot orientation in autumn and are very winter hardy [10]. Non-dormant cultivars have extensive shoot elongation with a vertical orientation in autumn and generally poor winter survival. Non-dormant cultivars are desirable because of higher shoot growth rates and faster maturity after cutting when compared to dormant cultivars [11, 12]. Therefore non-dormant cultivars could result in higher forage yield, and historically there has been interest in using less fall-dormant cultivars in regions with mild winters [13]. These FD-related differences in shoot growth rate and maturity might influence how alfalfa cultivars with contrasting FD respond to CF. Understanding the FD × CF interaction is very important to refine management and cutting schedule of cultivars differing in FD.

This research was undertaken to; the change of the forage quality in different phenological stages alfalfa cultivars which have different levels of dormancy is handled.

MATERIAL AND METHODS

The Research was carried out at University of Ankara, Faculty of Agriculture, experimental field of the Department of Field Crops that has altitude 860 m and lies between 39º 57' north latitude and the 32 º 52' east Longitude.

In the research, which is carried out in the field of investigation of field crops, in Ankara University Faculty of Agriculture, eight cultivars of alfalfa are used. These cultivars are divided in two and they are dormant and non-dormant cultivars. Dormant cultivars are; Bilensoy, Kayseri, Gözlü and Plato cultivars. Non-dormant cultivars are; Elçi, Mırna, Ma-414 and Posovina cultivars. Dormant cultivars are suitable for winter, while non-dormant cultivars are suitable for summer. These cultivars were named by numbers between 1 to 8 in all figures and tables. They were determined as 1: Bilensoy, 2: Gözlü, 3: Kayseri, 4: Plato, 5: Elçi, 6: Ma-414, 7: Mırna, 8: Posovina.

The average temperature during experimental season was 13.3 ºC. The long year's average of mean yearly temperature was 12 ºC. According to rainfall distribution of long years, 2007 has been dry and 2008 has been very dry as well. The soil of research area has clay and loamy structure. According to the analysis, the sample of soil had high-alkali and mid-calcareous structure. It was rich in potassium (192 kg da-1), total salt content in soil is 61%, poor in nitrogen (0.145%) and phosphorus (5.52 kg da-1) and insufficient in organic matter (1.05%) [14]. Meteorological values of the experimental area are presented in Table 1, 2 and 3.

The material used in the research was planted as 3 repetition in testing pattern of Coincidence Parcel in 10 March 2005. Parcel area is 5 m x 3.5 m = 17.5 m2. In each parcel 5 lines were placed in the manner that there is 70 cm in spaces. Seeds were planted by hand in the manner that 3 kg Alfalfa seeds were in one – tenth of a hectare. It is presented in table 4, phenological stages and cutting dates are indicated.

In these periods, grass samples representing 500 gr were taken from each sort and dried under 70 C for 24 hours until it reaches its constant weight. Dried samples were ground in 3 mm sieve and prepared to make their fiber analysis.

ADF, NDF

In this study, filter bag method was used in determining the cell wall components of coarse fodder, such as NDF and ADF. In the NDF analysis of coarse fodder used as research material, coarse fodder samples were weighed between 0.5-0.8 g, put into tare taken filter bags and the mouth of the bags were closed by pressing the heater. The bags on a plastic holder were placed in Ankom Fiber tank, 2 lt NDF solution (NDF solution includes: Ankom Neutral Detergent Dry powder – Ankom FND20C, Triethylene Glycol) and Alfa Amilaz were added into the tank and boiled at 100 ºC for 75 min. After boiling, the solution in the tank was discharged, the filter washed in hot pure water 2 or 3 times were taken plastic holder and washed in acetone for 3-5 min to remove the oil in coarse fodder. After washing process in acetone was repeated 2-3 times, the bags were dried first in ambient temperature for about 1 hour, then at 105 ºC for a night. Then the bags were weighed and %NDF components of the coarse fodder was calculated.

In the ADF analysis of coarse fodder according to the filter bag, like in NDF analysis, the bags weighed and placed on plastic holder have been placed in the tank. 2 lt ADF solution (ADF solution includes: Ankom Acid Detergent Dry powder "CTAB" - Ankom FAD20C, 1N H2SO4) have been added into the tank and boiled at 100 ºC for 60 min. After boiling process, the bags have been washed in the same way, weighed after dried, and %ADF components of the coarse fodder has been calculated.

TDN, DMI, DDM and RFV

The values are an indication of hay yield, total digestible nutrients (TDN), dry matter intake (DMI), digestible dry matter (DDM) and relative feed value (RFV), and were obtained following formulas by estimation method [15];

TDN = (-1.291 x ADF) + 101.35

DMI = 120% NDF % dry matter basis

DDM = 88.9-(0.779 x ADF % dry matter basis)

RFV = DDM% x DMI% x 0.775

Samples were analyzed for contents of ADF and NDF [16, 17]. The RFV is calculated based on the two laboratory determined parameters, NDF and ADF levels in a forage.

Table 1. Precipitation and Temperature for 1975-2008

	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec
Precipitation mm	41.8	36.9	38.7	49.0	51.2	35.4	14.5	10.9	18.5	30.2	33.9	46.9
Temperature ˚C	0.3	1.8	6.1	11.3	16.1	20.2	23.5	23.3	18.7	13.1	7.1	2.7

Reference: General Directorate of State Meteorology Affairs, Monthly Climatologic Observation Scale (Anonymous 2009b

Table 2. Precipitation, mm (2007-2008)

	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec
2007	39.0	16.4	37.5	23.8	17.9	31.7	3.9	9.8	0.0	19.7	66.7	44.4
2008	20.1	6.5	54.9	32.7	45.4	10.3	0.0	0.7	61.6	18.6	43.6	28.8

Reference: General Directorate of State Meteorology Affairs, Monthly Climatologic Observation Scale (Anonymous 2009b

Table 3. Temperature, °C (2007-2008)

	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec
2007	1.2	2.5	7.3	9.6	21.0	23.1	27.3	26.7	21.2	14.4	6.8	2.0
2008	-3.9	0.2	10.3	14.0	16.0	22.3	25.2	27.2	20.1	13.3	8.7	2.1

Reference: General Directorate of State Meteorology Affairs, Monthly Climatologic Observation Scale (Anonymous 2009b).

Table 4. Phenological Stages of Alfalfa (*Medicago sativa*) (2007-2008)

1rd Year	2rd Year	Phenological Stages
April 14, 2007	April 18, 2008	Vegetative, young growth
April 30, 2007	April 25, 2008	young growth; formation of flower Pre-buds
May 09, 2007	May 02, 2008	end of flower budding up to beginning of blooming
May 11, 2007	May 14, 2008	beginning of blooming (1/10 Bloom)
June 13, 2007	June 13, 2008	beginning of blooming up to full blooming

The NDF has been used as an indicator of forage intake because it takes into account all fiber components (lignin, cellulose and hemicellulose), the ADF has been used as an indicator of digestibility since it includes cellulose and lignin. Thus together, ADF and NDF take into account the most important traits of a forage, intake potential and digestibility, and are used to calculate RFV.

In experiment, there are 2 levels for year and 5 level for stage times. Properties obtained by the study were considered with analysis of variance in factorial order (SPSS.20) and Duncan's or LSD test was used to determine difference among the means of the different groups at P<0.05 and 0.01 levels of significance.

RESULT AND DISCUSSION

Acid Detergent Fiber (ADF)

In table 5, the inclination which is observed in ADF rate has occurred in a similar way with the NDF rate. In both experiment years, while Bilensoy cultivar has the minimum ADF rate with 25.385% and 25.778% in the first stage, an increase of ADF rates in all alfalfa cultivars is observed as the plant grows up. The maximum ADF rates have been reached in Plato cultivar with 35.297% in the fifth stage in 2008.

ADF and NDF responded similarly to CP, but in the opposite direction, with values increasing with increased FD score. Late stage time were dramatically higher in ADF than early or medium stage time. Non dormant varieties always had higher fiber concentration than the more dormant varieties (FD 3-4-5). Similar to the results for protein, Fall Dormancy of the variety had a greater effect in the early and mid-stage times compared with the late times [18].

The ADF concentration refers to the cell wall portions of the forage. These portions consist of cellulose and lignin. The ADF values are important because they describe the ability of an animal to digest the forage. As the ADF increases, the digestibility of the forage usually decreases [19]. The least digestible plant components, including cellulose and lignin. ADF values are inversely related to digestibility, so forages with low ADF concentrations are.

Table 5. Multiple comparisons results related to subgroups of years x stage time x cultivars in terms of ADF value.

Years	Cultivars	1rd stage	2rd stage	3rd stage	4rd stage	5rd stage
2007 (D)	1.Bilensoy	25.385±0.25 Cd$_a$	25.955±0.16 Cd$_b$	27.875±0.07 Cc$_a$	29.618±0.18 Eb$_a$	32.875±0.30 Ca$_b$
	2. Gözlü	25.875±0.17 BCd$_a$	26.450±0.18 Cd$_b$	28.288±0.23 Cc$_a$	31.012±0.18 Bb$_a$	33.412±0.23 BCa$_a$
	3. Kayseri	26.175±0.21 Bd$_a$	26.425±0.14 Cd$_b$	28.350±0.32 Cc$_a$	30.070±0.21 DEb$_b$	33.138±0.13 BCa$_b$
	4. Plato	27.295±0.20 Ae$_b$	28.455±0.18 Ad$_a$	30.358±0.29 Ac$_a$	32.045±0.30 Ab$_a$	34.895±0.29 Aa$_a$
2007 (ND)	5.Elçi	25.657±0.08 BCd$_a$	26.150±0.13 Cd$_b$	28.038±0.16 Cc$_a$	30.588±0.07 BCDb$_a$	32.925±0.13 Ca$_b$
	6. MA 414	26.025±0.20 BCe$_b$	27.185±0.18 Bd$_a$	29.087±0.29 Bc$_a$	30.775±0.30 BCb$_a$	33.625±0.30 Ba$_a$
	7. Mırna	26.295±0.21 Bd$_a$	26.545±0.14 Cd$_a$	28.470±0.32 BCc$_a$	30.190±0.21 CDEb$_a$	33.258±0.13 BCa$_a$
	8.Posovina	27.015±0.20 Ae$_b$	28.175±0.18 Ad$_a$	30.078±0.29 Ac$_a$	31.765±0.30 Ab$_a$	34.615±0.30 Aa$_a$
2008 (D)	1.Bilensoy	25.778±0.14 Ee$_a$	26.727±0.14 Cd$_a$	28.00±0.12 Dc$_a$	30.185±0.18 CDb$_a$	33.588±0.41 Ca$_a$
	2. Gözlü	26.458±0.13 BCDe$_a$	27.652±0.17 Bd$_a$	28.475±0.19 CDc$_a$	30.487±0.27 BCb$_a$	33.975±0.27 BCa$_a$
	3. Kayseri	26.380±0.19 CDEe$_a$	27.600±0.13 Bd$_a$	28.625±0.20 BCDc$_a$	30.913±0.21 Bb$_a$	34.313±0.18 Ba$_a$
	4. Plato	28.335±0.21 Ad$_a$	28.585±0.14 Ad$_a$	30.510±0.32 Ac$_a$	32.230±0.21 Ab$_a$	35.297±0.13 Aa$_a$
2008 (ND)	5.Elçi	26.028±0.12 DEe$_a$	27.318±0.18 BCd$_a$	28.050±0.05 Dc$_a$	29.738±0.25 Db$_b$	33.803±0.33 BCa$_a$
	6. MA 414	27.065±0.21 Bd$_a$	27.315±0.13 BCd$_a$	29.240±0.32 Bc$_a$	30.960±0.21 Bb$_a$	34.028±0.13 BCa$_a$
	7. Mırna	26.885±0.21 BCd$_a$	27.135±0.14 BCd$_a$	29.060±0.32 BCc$_a$	30.780±0.21 BCb$_a$	33.847±0.13 BCa$_a$
	8.Posovina	26.930±0.20 Ae$_b$	28.014±0.18 Ad$_a$	29.642±0.29 Ac$_a$	30.820±0.30 Ab$_a$	33.212±0.30 Aa$_a$

Capital letters were used in comparing cultivars in subgroups of year x stage time. (P<0.05) D: Dormant cultivars
Small letters were used in comparing stage time in subgroups of year x cultivars. (P<0.05 ND: Non-dormant cultivars
Subscripts were used in comparing years in subgroups of cultivars x stage time. (P<0.05)

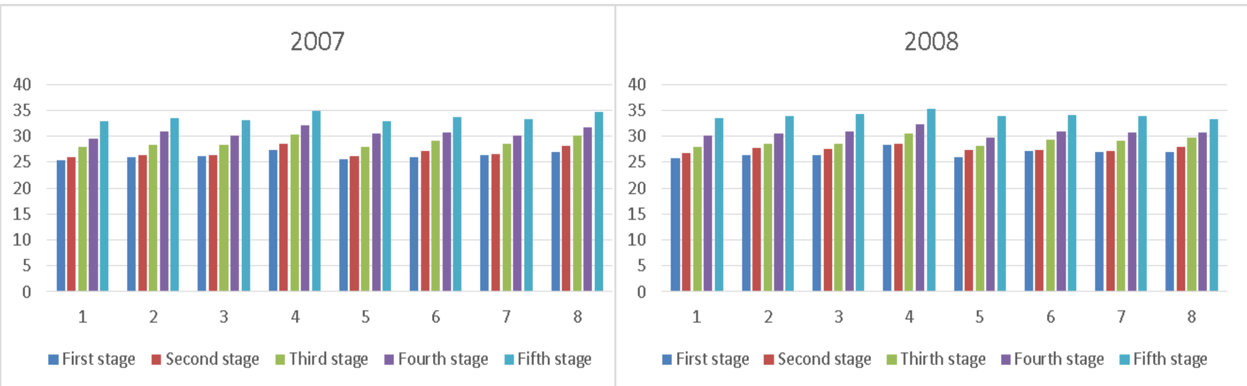

Figure 1. ADF's range of alfalfa cultivars and five different phenological stages in 2007-2008

Neutral Detergent Fiber (NDF)

In table 6, it is observed that the ternary interaction of year x stage time x cultivars is important statistically. It is determined that, there is a positive correlation between alfalfa maturement and NDF increase in both experiment years. In 2007 and 2008 experiment years, Plato cultivar has reached the top rate with 46.388% and 45.985% in the fifth stage. On the other hand, the minimal NDF rate has been measured in Posovina cultivar with 35.523% in the first stage in 2007.

Neutral detergent fiber values are important in ration formulation because they reflect the amount of forage that the animal can consume. As the NDF percentages increase, the dry matter intake will generally decrease. In general, low NDF values are desired because NDF increases as forages mature [19].

The timing of spring forage harvest is critical for obtaining optimal quality for animal production. For forage that serves as the primary fibre source in the diet, NDF is the principal forage quality variable of concern [20]. Some predictive equations can be used to estimate the forage quality of lucerne, assisting the producers in decision making at harvest time. An increased amount of NDF, ADF and ADL within the observed stages is in accordance with Coblentz et al. [21] and Elizalde et al. [22], Rinne et al. [23] observed an increasing content of NDF with increasing maturity of forage.

Dry Matter Intake (DMI)

As it is seen in the table 7, the binary interaction of year x stage time is remarkable in the level 5% statistically. Even if cultivar diversity has not been considered important, in 2007, the first year of investigation, Posovina cultivar had the maximum DMI rate, which is 3.378, has been obtained in the first stage. Plato cultivar had the minimum DMI rate, which is 2.587, has been measured during the fifth stage in 2008.

Table 6. Multiple comparisons results related to subgroups of years x stage time x cultivars in terms of NDF value

Years	Cultivars	1rd stage	2rd stage	3rd stage	4rd stage	5rd stage
2007 (D)	1.Bilensoy	35.633±0.17De$_{b}$	36.447±0.10 Dd$_{b}$	38.815±0.10 Fc$_{b}$	42.325±0.14 BCb$_{b}$	44.625±0.31 Da$_{b}$
	2. Gözlü	37.767±0.08 Be$_{a}$	38.395±0.13 Bd$_{b}$	40.207±0.23 Bc$_{b}$	42.780±0.10 ABb$_{a}$	45.113±0.07 BCa$_{b}$
	3. Kayseri	36.473±0.17 Ce$_{b}$	37.287±0.10 Cd$_{b}$	39.655±0.10 CDc$_{b}$	43.165±0.14 Ab$_{b}$	45.465±0.31 Ba$_{b}$
	4. Plato	38.385±0.20 Ae$_{b}$	39.545±0.10 Ad$_{a}$	41.447±0.29 Ac$_{a}$	43.135±0.30 Ab$_{a}$	45.985±0.30 Aa$_{a}$
2007 (ND)	5.Elçi	37.638±0.08 Be$_{a}$	38.130±0.13 Bd$_{b}$	40.017±0.16 BCc$_{a}$	42.567±0.07 BCb$_{a}$	44.905±0.13 CDa$_{b}$
	6. MA 414	36.698±0.08 Ce$_{a}$	37.325±0.13 Cd$_{b}$	39.137±0.23 EFc$_{b}$	41.710±0.10 Db$_{a}$	44.043±0.07Ea$_{b}$
	7. Mırna	36.362±0.17 Ce$_{b}$	37.178±0.10 Cd$_{b}$	39.545±0.10 DEc$_{b}$	43.055±0.14 Ab$_{b}$	45.355±0.30 BCa$_{b}$
	8.Posovina	35.523±0.17 De$_{b}$	36.337±0.18 Dd$_{b}$	38.705±0.10 Fc$_{b}$	42.215±0.14 Cb$_{b}$	44.51±0.31Da$_{b}$
2008 (D)	1.Bilensoy	36.483±0.17 De$_{a}$	37.297±0.10 Dd$_{a}$	39.665±0.10 DEc$_{a}$	43.175±0.14 Bb$_{a}$	45.475±0.31 DEa$_{a}$
	2. Gözlü	38.138±0.12 Be$_{a}$	39.718±0.18 Ad$_{a}$	41.190±0.17 Ac$_{a}$	41.847±0.25 Cb$_{b}$	45.912±0.33 BCDa$_{a}$
	3. Kayseri	37.322±0.17Ce$_{a}$	38.137±0.15 Cd$_{a}$	40.505±0.10 Bc$_{a}$	44.015±0.14 Ab$_{a}$	46.315±0.31 ABa$_{a}$
	4. Plato	39.425±0.21 Ad$_{a}$	39.675±0.10 Ad$_{a}$	41.600±0.32 Ac$_{a}$	43.320±0.21Bb$_{a}$	46.388±0.13 Aa$_{a}$
2008 (ND)	5.Elçi	38.008±0.12 Be$_{a}$	39.297±0.10 Ad$_{a}$	40.030±0.05 CDc$_{a}$	41.717±0.25 Cb$_{b}$	45.782±0.33 CDEa$_{a}$
	6. MA 414	37.067±0.12 Ce$_{a}$	38.648±0.10 Bd$_{a}$	40.120±0.17 BCDc$_{a}$	40.777±0.25 Db$_{b}$	44.842±0.33 Fa$_{a}$
	7. Mırna	37.212±0.17 Ce$_{a}$	38.027±0.15 Cd$_{a}$	40.395±0.10 BCc$_{a}$	43.905±0.14 Ab$_{a}$	46.205±0.31 ABCa$_{a}$
	8.Posovina	36.373±0.17 De$_{a}$	37.188±0.14 Dd$_{a}$	39.555±0.10 Ec$_{a}$	43.065±0.14Bb$_{a}$	45.365±0.31 Ea$_{a}$

Capital letters were used in comparing cultivars in subgroups of year x stage time. (P<0.05) D: Dormant cultivars
Small letters were used in comparing stage time in subgroups of year x cultivars. (P<0.05) ND: Non-dormant cultivars
Subscripts were used in comparing years in subgroups of cultivars x stage time. (P<0.05)

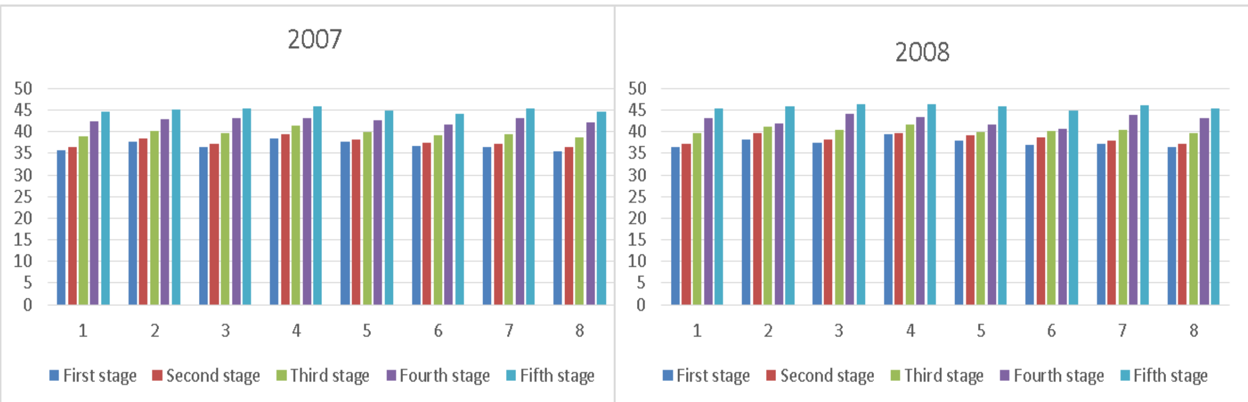

Figure 2. NDF's range of alfalfa cultivars and five different phenological stages in 2007-2008.

Table 7. Multiple comparisons results related to subgroups of years x stage time in terms of DMI value

Years	Cultivars	1rd stage	2rd stage	3rd stage	4rd stage	5rd stage
2007 (D)	1.Bilensoy	3.367±0.016 Aa$_a$	3.293±0.009 Ab$_a$	3.092±0.008 Bc$_a$	2.835±0.009 ABd$_a$	2.689±0.018 ABCe$_a$
	2. Gözlü	3.177±0.007 Ca$_a$	3.126±0.011 Cb$_a$	2.985±0.017 Ac$_a$	2.805±0.007 BCd$_b$	2.660±0.004BCDe$_a$
	3. Kayseri	3.290±0.015 Ba$_a$	3.218±0.009 Bb$_a$	3.026±0.007 CDc$_a$	2.780±0.009 Cd$_a$	2.640±0.017 DEe$_a$
	4. Plato	3.127±0.016 Da$_a$	3.035±0.014 Db$_a$	2.896±0.020 Ec$_a$	2.782±0.019 Cd$_a$	2.609±0.017 Ee$_a$
2007 (ND)	5.Elçi	3.188±0.007 Ca$_a$	3.147±0.011 Ca$_a$	2.999±0.012 Db$_a$	2.819±0.005BCc$_b$	2.672±0.008 BCDd$_a$
	6. MA 414	3.270±0.007 Ba$_a$	3.215±0.011 Bb$_a$	3.066±0.018 BCc$_a$	2.877±0.007 Ad$_b$	2.724±0.004 Ae$_a$
	7. Mırna	3.30±0.015Ba$_a$	3.228±0.009 Bb$_a$	3.035±0.008 CDc$_a$	2.787±0.009 Cd$_a$	2.646±0.018 CDEe$_a$
	8.Posovina	3.378±0.016 Aa$_a$	3.302±0.009 Ab$_a$	3.100±0.008 Bc$_a$	2.842±0.009 ABd$_a$	2.696±0.019 ABe$_a$
2008 (D)	1.Bilensoy	3.290±0.015 Aa$_b$	3.217±0.009 Ab$_b$	3.025±0.007 Ac$_b$	2.780±0.009 Cd$_b$	2.639±0.018 ABCe$_b$
	2. Gözlü	3.147±0.010 Ca$_a$	3.022±0.011 Db$_b$	2.914±0.012 Cc$_b$	2.867±0.017 Bc$_a$	2.614±0.019 BCDd$_b$
	3. Kayseri	3.215±0.014 Ba$_b$	3.147±0.008 BCb$_b$	2.963±0.007 Bc$_b$	2.726±0.008 Dd$_b$	2.591±0.017 De$_b$
	4. Plato	3.044±0.016 Da$_b$	3.025±0.011Da$_a$	2.885±0.023 Cb$_a$	2.770±0.014 CDc$_a$	2.587±0.007 Dd$_a$
2008 (ND)	5.Elçi	3.157±0.010 Ca$_a$	3.054±0.014 Db$_b$	2.998±0.003 ABc$_a$	2.877±0.017 Bd$_a$	2.622±0.019 BCDe$_b$
	6. MA 414	3.237±0.011 Ba$_a$	3.105±0.012 Cb$_b$	2.991±0.013 ABc$_b$	2.943±0.018Ad$_a$	2.677±0.020 Ae$_a$
	7. Mırna	3.225±0.014 Ba$_b$	3.156±0.008 Bb$_b$	2.971±0.007 Bc$_b$	2.733±0.008 Dd$_b$	2.598±0.017CDe$_b$
	8.Posovina	3.299±0.015 Aa$_b$	3.228±0.009 Ab$_b$	3.034±0.008 Ac$_b$	2.786±0.009 Cd$_b$	2.645±0.018 ABe$_a$

Capital letters were used in comparing cultivars in subgroups of year x stage time. ($P<0.05$) D: Dormant cultivars
Small letters were used in comparing stage time in subgroups of year x cultivars. ($P<0.05$) ND: Non-dormant cultivars
Subscripts were used in comparing years in subgroups of cultivars x stage time. ($P<0.05$)

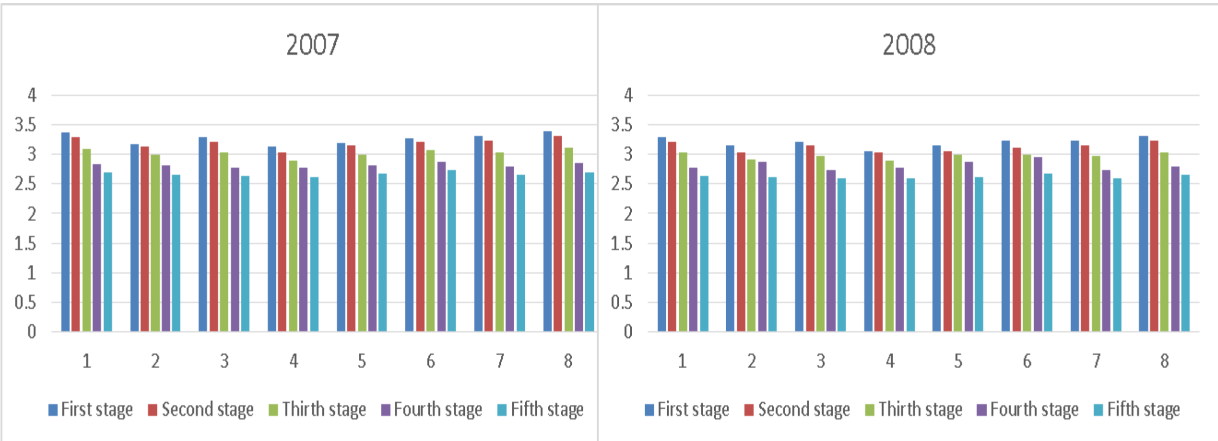

Figure 3. DMI's range of alfalfa cultivars and five different phenological stages in 2007-2008.

Digestible Dry Matter (DDM)

As is seen in the table 8, the binary interaction of year x stage time and DDM can be considered as remarkable statistically in the level of 5%. Whereas the maximum DDM rate, which is 69.125%, has been obtained in Bilensoy cultivar in the first stage of 2007, the minimum DDM rate, which is 61.403% has been obtained in Plato cultivar in the fifth stage of 2008.

Total Digestible Nutrients (TDN)

In table 9, the ternary interaction of year x cultivars x stage time on TDN value, which is 5% has been found remarkable, as it is seen on DDM value statistically. While the maximum Maximum TDN rate, which is 68,578%, has been obtained in Bilensoy cultivar in the first stage of 2007, the minimum TDN rate, which is 55.781% has been obtained in Plato cultivar in the fifth stage.

Table 8. Multiple comparisons results related to subgroups of years x cultivars x stage time in terms of DDM

Years	Cultivars	1rd stage	2rd stage	3rd stage	4rd stage	5rd stage
2007 (D)	1.Bilensoy	69.125±0.192 Aa$_a$	68.681±0.127 Aa$_a$	67.185±0.052 Ab$_a$	65.828±0.136 Ac$_a$	63.290±0.231 Ad$_a$
	2. Gözlü	68.743±0.130 ABa$_a$	68.295±0.143 Aa$_a$	66.864±0.180 Ab$_a$	64.741±0.143 Dc$_a$	62.872±0.179ABd$_a$
	3. Kayseri	68.510±0.163 Ba$_a$	68.315±0.108 Aa$_a$	66.815±0.249 Ab$_a$	65.475±0.166 ABc$_a$	63.086±0.100 ABd$_a$
	4. Plato	67.637±0.153 Ca$_a$	66.734±0.143 Cb$_a$	65.252±0.224 Cc$_a$	63.937±0.232 Ed$_a$	61.717±0.232 Ce$_a$
2007 (ND)	5.Elçi	68.913±0.063 ABa$_a$	68.529±0.103 Aa$_a$	67.059±0.123 Ab$_a$	65.072±0.533 BCDc$_b$	63.251±0.099 Ad$_a$
	6. MA 414	68.627±0.153 ABa$_a$	67.723±0.143 Bb$_a$	66.241±0.224 Bc$_a$	64.926±0.232 CDd$_a$	62.706±0.232Be$_a$
	7. Mırna	68.416±0.163 Ba$_a$	68.221±0.108 Aa$_a$	66.722±0.249 Ab$_a$	65.382±0.166 ABCc$_a$	62.992±0.100 ABd$_a$
	8.Posovin a	67.855±0.153 Ca$_a$	66.952±0.143Cb$_a$	65.470±0.224 Cc$_a$	64.155±0.232 Ed$_a$	61.935±0.232 Ce$_a$
2008 (D)	1.Bilensoy	68.819±0.113Aa$_a$	68.079±0.106 Ab$_b$	67.088±0.100 Ac$_a$	65.386±0.141 ABd$_a$	62.735±0.316 Ae$_b$
	2. Gözlü	68.290±0.101 ABa$_a$	67.359±0.128 Bb$_b$	66.718±0.150 ABc$_a$	65.150±0.214 BCd$_a$	62.433±0.211 ABe$_a$
	3. Kayseri	68.350±0.149 ABa$_a$	67.400±0.104 Bb$_b$	66.601±0.155 ABCc$_a$	64.819±0.160 Cd$_b$	62.171±0.143 Be$_b$
	4. Plato	66.827±0.163 Ca$_b$	66.632±0.108 Ca$_a$	65.133±0.249 Db$_a$	63.793±0.166 Dc$_a$	61.403±0.100 Cd$_a$
2008 (ND)	5.Elçi	68.625±0.100 Aa$_a$	67.620±0.139 ABb$_b$	67.049±0.036 A$_a$	65.734±0.196 A$_a$	62.568±0.258 AB$_b$
	6. MA 414	67.816±0.163 Bab$_b$	67.622±0.108 ABa$_a$	66.122±0.249 Cb$_a$	64.782±0.166 Cc$_a$	62.393±0.100 ABd$_a$
	7. Mırna	67.957±0.163 ABa$_a$	67.762±0.108 ABa$_a$	66.262±0.249 BCb$_a$	64.922±0.166 BCc$_a$	62.533±0.100 ABd$_a$
	8.Posovin a	67.045±0.163 Ca$_b$	66.850±0.108 Ca$_a$	65.351±0.249 Db$_a$	64.011±0.166 Dc$_a$	61.621±0.100 Cd$_a$

Capital letters were used in comparing cultivars in subgroups of year x stage time. (P<0.05) D: Dormant cultivars
Small letters were used in comparing stage time in subgroups of year x cultivars. (P<0.05) ND: Non-dormant cultivars
Subscripts were used in comparing years in subgroups of cultivars x stage time. (P<0.05)

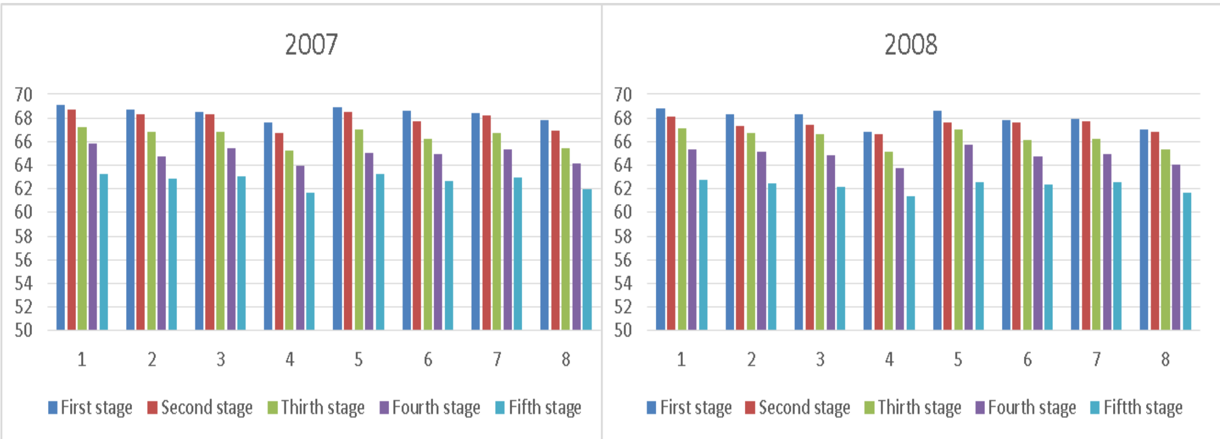

Figure 4. DDM's range of alfalfa cultivars and five different phenological stages in 2007-2008.

The TDN refers to the nutrients that are available for livestock. This variable is related to the ADF concentration of the forage. As ADF increases, TDN declines. As a result, animals are unable to utilize the nutrients that are present in the forage [15]. In the present study, pure alfalfa (56.64%) and binary mixtures of alfalfa + grasses had the highest TDN values (53.53 to 54.28%) whereas pure grasses had the lowest values (44.28 to 46.30%) (Average of two years) (Tab. 4).

Relative Feed Value (RFV)

The RFV is an index that is used to predict the intake and energy value of forages. This index is derived from the DDM and dry matter intake DMI. Forages with an RFV value over 151, between 150-125, 124-103, 102-87, 86-75, and less than 75 are categorized as prime, premium good, fair, poor and rejected, respectively [24].

In table 10, the ternary interaction of year x cultivars x stage time on RFV value can be considered important in the level of 5%, as it is on both DDM and TDN value. While the maximum RFV value, which is 180.47%, has been obtained in Bilensoy cultivar in the first stage, the minimum RFV value, which is 123.14% has been obtained in Plato cultivar in the fifth stage of 2008.

The relative is not a direct measure of the nutritional content of forage, but it is important for estimating the value of forage [25].

Table 9. Multiple comparisons results of years x cultivars x stage time subgroups in regard to TDN value.

Years	Cultivars	1rd stage	2rd stage	3rd stage	4rd stage	5rd stage
2007 (D)	1.Bilensoy	68.578±0.139 Aa$_a$	67.842±0.210 Aa$_a$	65.363±0.086 Ab$_a$	63.114±0.226 Ac$_a$	58.908±0.382 Ad$_a$
	2. Gözlü	67.945±0.215 ABa$_a$	67.203±0.237 Aa$_a$	64.831±0.299 Ab$_a$	61.313±0.238 Dc$_a$	58.214±0.296 ABd$_a$
	3. Kayseri	67.558±0.269 Ba$_a$	67.235±0.180 Aa$_a$	64.750±0.412 Ab$_a$	62.530±0.274 ABCc$_a$	58.569±0.165 ABd$_a$
	4. Plato	66.112±0.253 Ca$_a$	64.615±0.238 Cb$_a$	62.158±0.371 Cc$_a$	59.980±0.384 Ed$_a$	56.301±0.384 Ce$_a$
2007 (ND)	5.Elçi	68.226±0.105 ABa$_a$	67.590±0.171 Aa$_a$	65.154±0.203 Ab$_a$	61.862±0.088 BCDc$_b$	58.844±0.164 Ad$_a$
	6. MA 414	67.752±0.253 ABa$_a$	66.254±0.238 Bb$_a$	63.798±0.371Bc$_a$	61.619±0.384 CDd$_a$	57.940±0.384 Be$_a$
	7. Mırna	67.403±0.269 Ba$_a$	67.080±0.180 Aa$_a$	64.595±0.412 Ab$_a$	62.375±0.274 ABCc$_a$	58.415±0.165 ABd$_a$
	8.Posovina	66.474±0.253 Ca$_a$	64.976±0.238 Cb$_a$	62.520±0.371 Cc$_a$	60.341±0.384 Ed$_a$	56.662±0.384 Ce$_a$
2008 (D)	1.Bilensoy	68.071±0.186 Aa$_a$	66.845±0.175 Ab$_b$	65.202±0.151 Ac$_a$	62.381±0.233 ABd$_a$	57.989±0.523 Ae$_b$
	2. Gözlü	67.193±0.167 BCDa$_a$	65.651±0.213 Bb$_b$	64.589±0.249 ABc$_a$	61.991±0.354 BCd$_a$	57.488±0.350 ABe$_a$
	3. Kayseri	67.293±0.247 ABCa$_a$	65.718±0.173 Bb$_b$	64.395±0.256 ABCc$_a$	61.442±0.265 Cd$_b$	57.053±0.238 Be$_b$
	4. Plato	64.770±0.269 Ea$_b$	64.447±0.180 Ca$_a$	61.962±0.412 Db$_a$	59.741±0.274 Dc$_a$	55.781±0.165 Cd$_a$
2008 (ND)	5.Elçi	67.748±0.160ABa$_a$	66.083±0.230 ABb$_b$	65.137±0.060 Ac$_a$	62.959±0.324 Ad$_a$	57.711±0.428 ABe$_b$
	6. MA 414	66.409±0.269 Da$_b$	66.086±0.180 ABa$_a$	63.601±0.412 Cb$_a$	61.381±0.274 Cc$_a$	57.420±0.165 ABd$_a$
	7. Mırna	66.641±0.269 Da$_a$	66.319±0.180 ABa$_a$	63.834±0.412 BCb$_a$	61.613±0.274 BCc$_a$	57.653±0.165 ABd$_b$
	8.Posovina	65.131±0.269 Ea$_b$	64.808±0.180 Ca$_a$	62.323±0.412 Db$_a$	60.103±0.274 Dc$_a$	56.142±0.165 Cd$_a$

Capital letters were used in comparing cultivars in subgroups of year x stage time. (P<0.05) D: Dormant cultivars
Small letters were used in comparing stage time in subgroups of year x cultivars. (P<0.05) ND: Non-dormant cultivars
Subscripts were used in comparing years in subgroups of cultivars x stage time. (P<0.05)

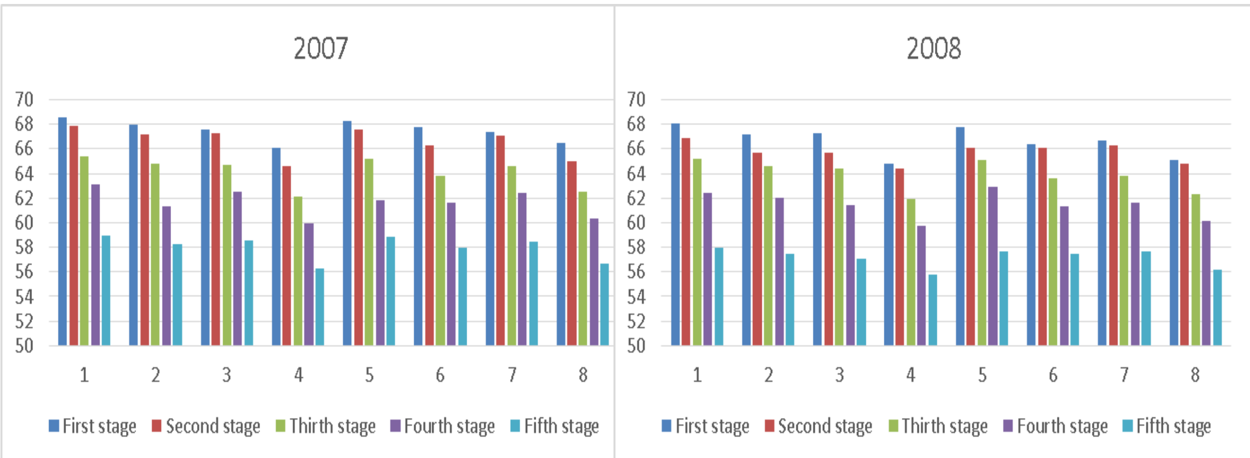

Figure 5. TDN's range of alfalfa cultivars and five different phenological stages in 2007-2008.

Table 10. Multiple comparisons results of years x cultivars x stage time subgroups in regard to RFV value.

Years	Cultivars	1rd stage	2rd stage	3rd stage	4rd stage	5rd stage
2007 (D)	1.Bilensoy	180.47 Aa_a	175.30 Ab_a	161.02 Ac_a	144.69 Ad_a	131.96 ABe_a
	2. Gözlü	169.32 Da_a	165.47 Db_a	154.72 Cc_a	140.78 Bd_b	129.65 BCe_a
	3. Kayseri	174.74 Ca_a	170.43 Bb_a	156.74 BCc_a	141.11 Bd_a	129.10 Ce_a
	4. Plato	163.93 Ea_a	156.99 Eb_a	146.48 Dc_a	137.91 Cd_a	124.87 De_a
2007 (ND)	5.Elçi	170.33 Da_a	167.19 CDb_a	155.89 BCc_a	142.21 Bd_b	131.03 $ABCe_a$
	6. MA 414	173.96 Ca_a	168.79 BCb_a	157.47 Bc_a	144.80 Ad_b	132.44 Ae_a
	7. Mırna	175.03 Ca_a	170.70 Bb_a	156.96 BCc_a	141.27 Bd_a	129.22 Ce_a
	8.Posovina	177.71 Ba_a	171.40 Bb_a	157.36 Bc_a	141.38 Bd_a	129.45 BCe_a
2008 (D)	1.Bilensoy	175.49 Aa_b	169.80 Ab_b	157.34 Ac_b	140.88 Cd_b	128.36 ABe_b
	2. Gözlü	166.57 Da_b	157.77 EFb_b	150.68 Dc_b	144.85 Bd_a	126.53 BCe_b
	3. Kayseri	170.37 BCa_b	164.40 CDb_b	152.96 CDc_b	137.00 Dd_b	124.89 CDe_b
	4. Plato	157.70 Ea_b	156.24 Fa_a	145.68 Eb_a	137.00 Dc_a	123.14 Dd_a
2008 (ND)	5.Elçi	167.97 CDa_a	160.08 Eb_b	155.81 ABc_a	146.60 ABd_a	127.16 $ABCe_b$
	6. MA 414	170.19 BCa_b	162.77 Db_b	153.32 $BCDc_b$	147.81 Ad_a	129.46 Ae_b
	7. Mırna	169.88 BCa_b	165.76 BCb_b	152.60 CDc_b	137.56 Dd_b	125.92 BCe_b
	8.Posovina	171.48 Ba_b	167.23 Bb_b	153.70 BCc_b	138.27 Dd_b	126.38 BCe_b

Capital letters were used in comparing cultivars in subgroups of year x stage time. (P<0.05) D: Dormant cultivars
Small letters were used in comparing stage time in subgroups of year x cultivars. (P<0.05) ND: Non-dormant cultivars
Subscripts were used in comparing years in subgroups of cultivars x stage time. (P<0.05)

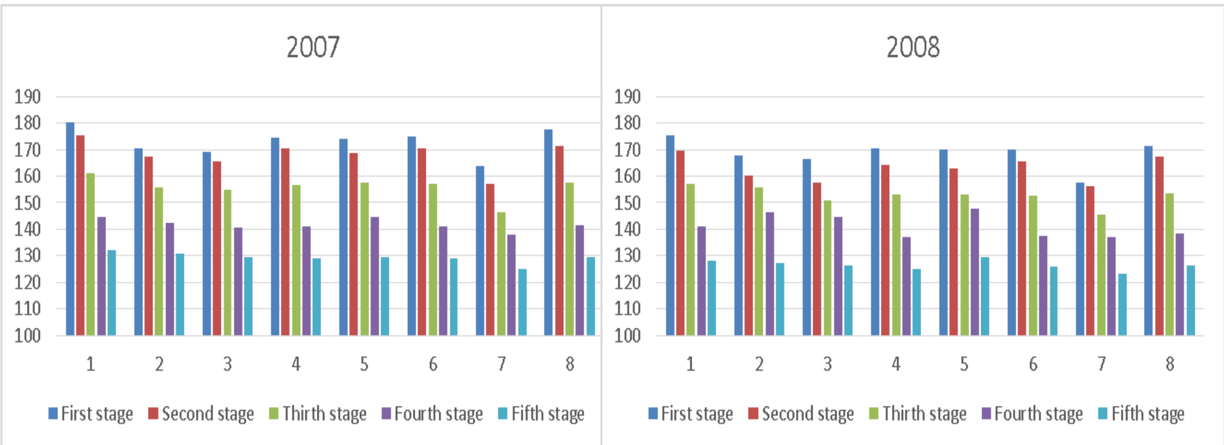

Figure 6. RFV's range of alfalfa cultivars and five different phenological stages in 2007-2008

CONCLUSIONS

Phenological stages had a more powerful effect on both yield and quality than did variety. Choice of higher quality (low FD) varieties reduced but did not overcome the negative effect of late stage time on quality.

Cutting at the pre-bud and bud stage produces a higher quality forage than at later stages, but repeatedly cutting at early stages reduces root reserves which results in poor stands and lower yields. Cutting when regrowth at the crown appears and at one-tenth bloom maximizes forage yield, quality and benefits stand longevity. The last cutting of the year may determine how well the alfalfa performs the next year. The last cutting before fall dormancy should allow four to five weeks of growth so that root reserves are replenished.

Forage quality and stand persistence are affected by the stage times which is chosen. Cutting for high quality will reduce total season yield, so one must ensure that the high quality will produce a return to offset the yield loss. Early season growth may not flower normally and quality will decline if it doesn't. Therefore, using a forage quality stick (available from some state forage associations and some alfalfa seed marketing companies), or measuring forage height and plant stage (as described later) is crucial in determining when to do the first stage time in order to harvest alfalfa of the desired quality. The stage to cut alfalfa for optimum forage quality for dairy cattle ranges from the vegetative to early bud stage on first cutting and is generally at bud stage on later cutting. Later stages may be harvested for animal's nutritional requirements.

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