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

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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)

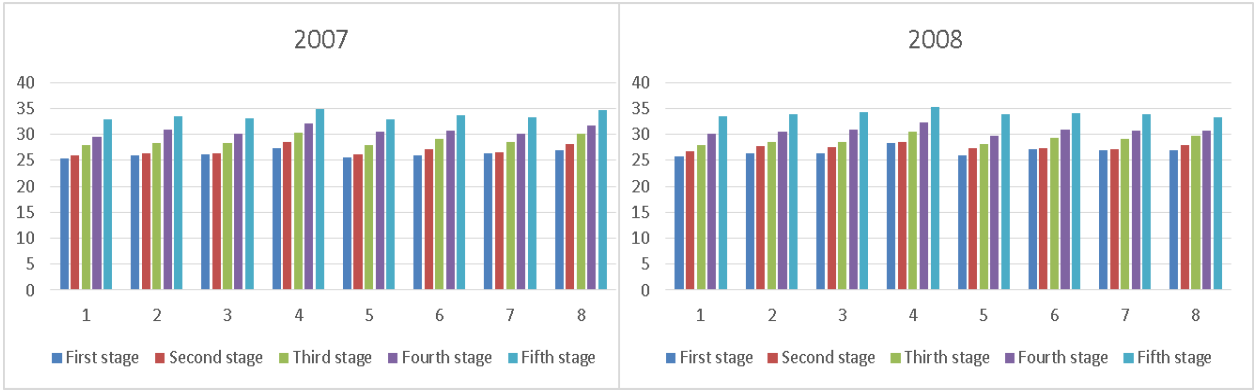

Figure 1. ADF's range of alfalfa cultivars and five different phenological stages in 2007-2008

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)

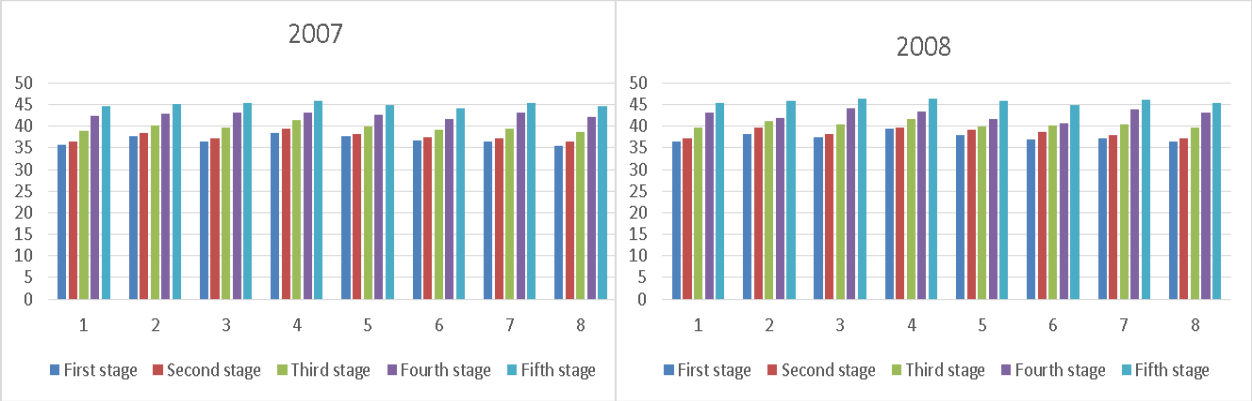

Figure 2. NDF's range of alfalfa cultivars and five different phenological stages in 2007-2008.

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±0.004$BCDe_a$
	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)

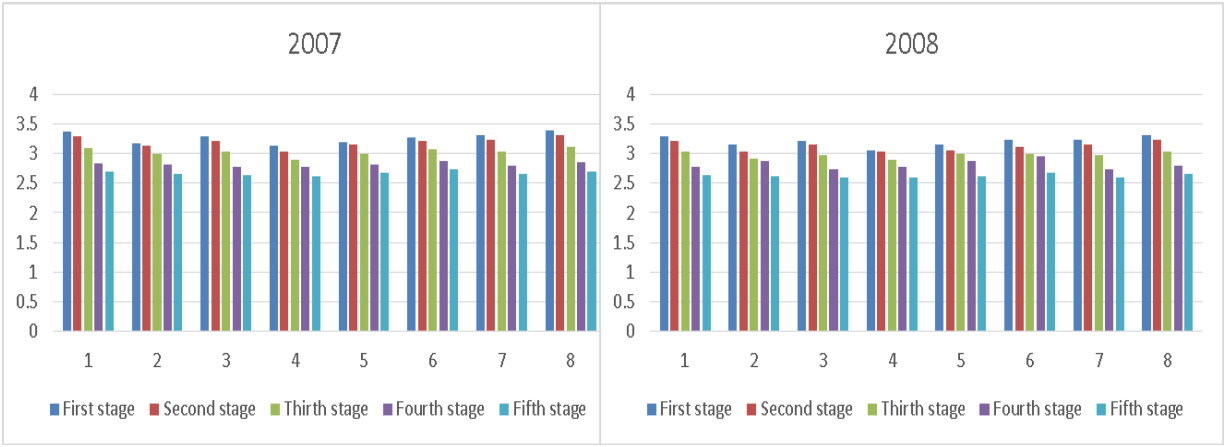

Figure 3. DMI's range of alfalfa cultivars and five different phenological stages in 2007-2008.

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.Posovina	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.Posovina	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)

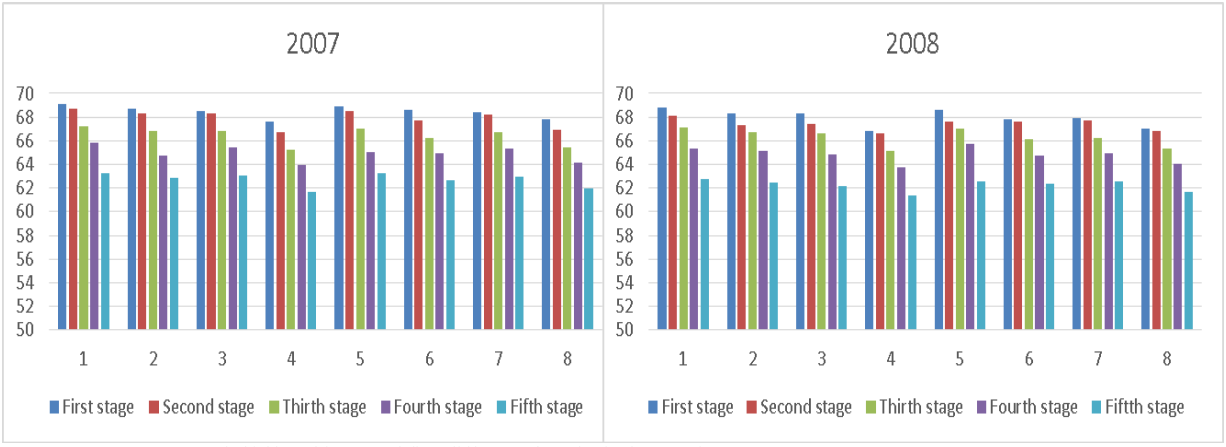

Figure 4. DDM's range of alfalfa cultivars and five different phenological stages in 2007-2008.

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 ABc_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)

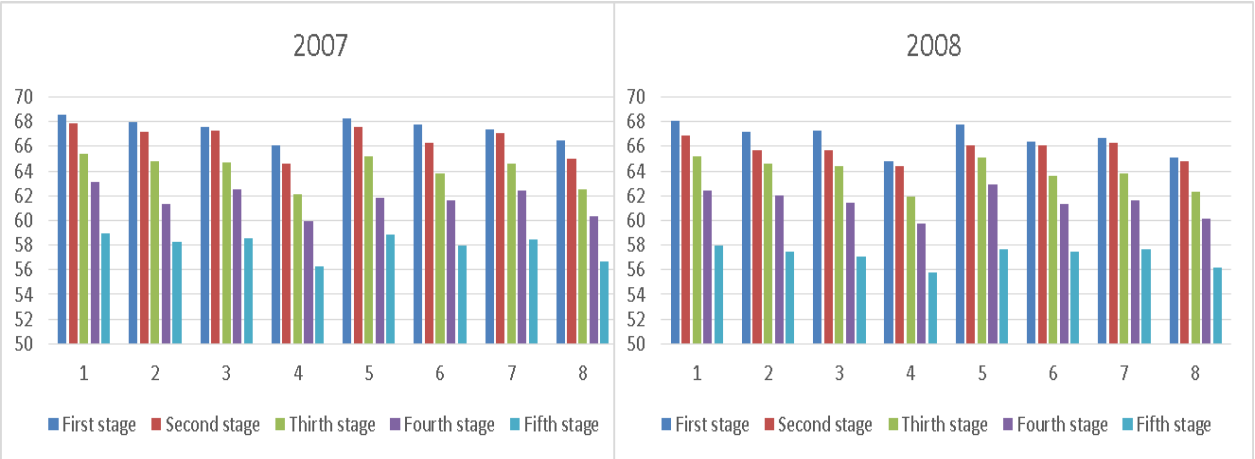

Figure 5. TDN's range of alfalfa cultivars and five different phenological stages in 2007-2008.

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)

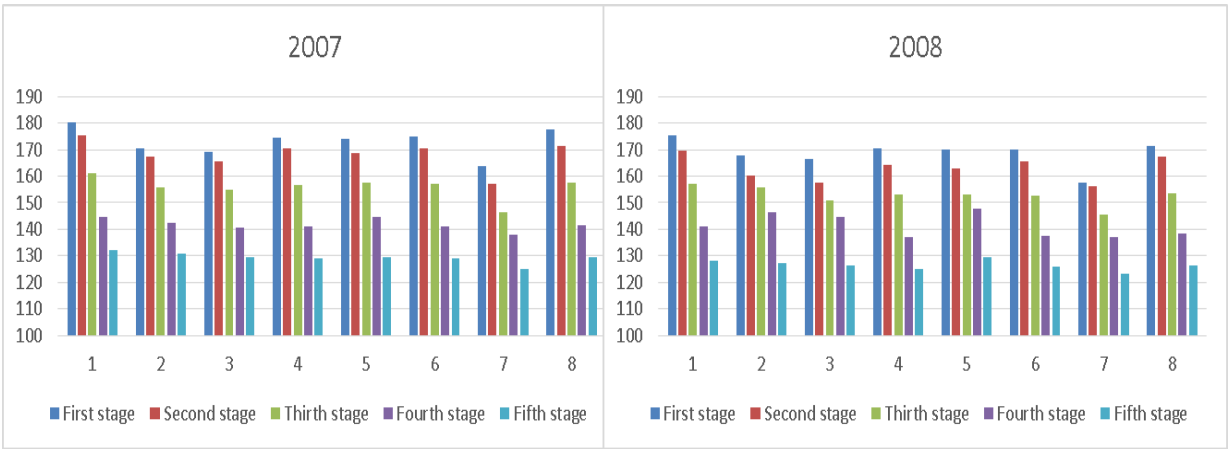

Figure 6. RFV's range of alfalfa cultivars and five different phenological stages in 2007-2008

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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