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Investigation of feed value of alfalfa (*Medicago sativa* L.) harvested at different maturity stages

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ABSTRACT

The aim of this study was to determine the green herbage and dry herbage yield, chemical and nutritional values and RFV of alfalfa (*Medicago sativa* L.) at different stages of maturity under Isparta ecological conditions in 2012. To achieve this goal random sampling plots was created according to an experimentation pattern, and the number of harvests was determined according to regional conditions. In chemical analysis, the following samples were analyzed for Dry Matter (DM), Crude Protein (CP), Ether Extract (EE), Ash, Crude Fiber (CF), Nitrogen Free Extract (NFE), Notral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF), as well as macro and micro minerals. The highest total green herbage yield was 9609.33 kg/da during the budding period, which decreased by 28% with maturity. The highest total dry matter yield, which depends on hay yield, was determined as 1742.63 kg/da at the beginning of the flowering period. The highest CP was determined during the budding period and decreased with maturity. DM, CS, NDF and ADF content increased while ash, Digestible Dry Matter (DDM), Total Digestible Nutrients (TDN), Dry Matter Intake (DMI), Metabolisable Energy (ME), Net Energy Lactation (NEL), and Digestible Energy (DE) content decreased with maturity. The highest RFV was determined during budding and the beginning of the flowering periods. Taking both yield and feed value results into consideration based on Isparta's conditions, the best harvesting period of alfalfa was determined to be at the beginning of the flowering period.

Key words: Alfalfa, Dynamics of cell wall content, Feed value, Forage yield, Macro and micro mineral content.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is one of the most important forage plants; it is cultivated on all the continents, and hay making is the most important method of conservation. The nutritive value of alfalfa forage depends on the cultivar and many environmental factors (Alibés *et al*., 1991). Because of the rich vitamin resources in its green herbage and its high protein yield per unit area, and because animals like to eat it green or as hay, alfalfa has been called the queen of feed crops. It provides a large amount of soluble protein with rapid transition from the intestinal tract and the capability of synthesis by rumen microorganisms. Therefore, alfalfa hay is an important protein source for animals. Alfalfa is an essential forage crop due to its nutrition value: it contains each of the protective nutrient vitamins E, A and K or their precursors, enables the synthesis of vitamin B and stimulates cellulose digestion, and has a lower cell wall content than other forages (Tomic *et al*., 2001). Besides all of these features, alfalfa is also an economic plant due to its high quality products, enabling multiple harvests in a year and because it is a perennial. It is possible to get a high yield per unit area after a good facility at low cost in the long term. The nutrition value of alfalfa hay varies according to different harvesting and maturation times. The aim of this project was to determine the green and dry herbage yields and the chemical and nutritional values of alfalfa (*Medicago sativa* L.) at different stages of maturity (budding period, beginning of flowering period, full blooming period and seed setting period) under Isparta ecological conditions in 2012.

MATERIALS AND METHODS

The experiment area at Suleyman Demirel University in Isparta, Turkey was divided into random sampling plots according to the experimentation pattern. A fence surrounded the 240 square meter harvest plot. The plots were harvested in 2012 in the budding period on 8 May, 22 June, 13 July, 6 August and 17 September, at beginning of the flowering period on 31 May, 25 June, 27 July, 25 August, 18 October, in the full blooming period on 7 June, 6 July, 10 August, 3 September, and in the seed setting period on 3 July, 13 August, 4 October. The plots were

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"This work is based on my thesis from Suleyman Demirel University, which has not been traditionally published"

harvested to 5 cm stubble height using a plot harvest machine. The number of harvests was determined by regional conditions. A sampling quadrant of 1m×1m was created by using wooden slats were thrown to three replications x 3 to determine the forage and dry matter yield. After the harvest, samples were collected, hand separated, dried at 70°C for 48 hrs, and weighed. The dried samples were reassembled and ground to pass through a 1-mm screen. Crude nutrients and crude fiber contents were determined by the Weende method (AOAC, 1984) and ADF (acid detergent fiber) and NDF (neutral deter-gent fiber) concentrations were measured according to standard laboratory procedures for forage quality analysis (Anonymous, 2010). NEL and ME were determined by the methods (TSE, 1991) and (Kirchgessner and Keller, 1981), respectively. The quality of the forages was esti-mated according to the following equations based on crude nutrition analysis (Logan *et al*., 1976; Rohweder *et al*., 1976a; Rohweder *et al.*, 1976b; Rohweder *et al.*, 1978).

Totally Digestible Nutrients (TDN), % = 82.38-(.7515x %ADF)

Digestibility Dry Matter (DDM), % = 88,9 - (.779 x %ADF)

Dry Matter Intake (DMI), BW% =120 /%NDF

Metabolisable Energy-ADF (ME_{ADF} MJ/kg DM)=14.70-0.150*ADF

Metabolisable Energy-NDF (ME_{NDF} kcal/kg DM)=3381.9-19.98*NDF

Net Energy Lactation (NEL_{ADF} MJ/kg DM) = 9.23-0.105*ADF

Digestible Energy (DE Mcal/kg^{-1} DM)= 0.27+0.0428*SKM

Relative Feed Value (RFV)=DDM x DMI / 1.29

Repeated measurement analysis of variance (ANOVA) was carried out to compare chemical composition, herbage and dry herbage yield and crude protein disappearance, using SPSS 18. Significance between individual maturity means was identified using Tukey's multiple range test (Pearse and Hartley, 1966). Mean differences were considered significant at $P<0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance. All experimental procedures were in accordance with established standards for the care and use of animals for research purposes. The experiment was conducted under the protocol, which was approved by Suleyman Demirel University Animal Use Local Ethical Committee (No:2012-01-10).

RESULTS AND DISCUSSION

Economic incentives dictate that alfalfa producers carefully consider the impact of harvest management on yield and nutritive value. Chemical composition of forage depends on plant characteristics and harvesting and storage methods. During the spring and early summer, DM is being produced and nutritive value is generally declining more rapidly. When dry matter yield, leafstalk percentage increase, leaf percentage decrease with advancing maturity. The percentage of leaf is a sign of the quality and palatable forage. Alfalfa quality declines with advancing maturity. Generally, CP content of forage is positively, fiber content of forages is inversely related with quality. Producing more forage is important in terms of animal nutrition, high protein content is important in terms of quality. There is inverse relation between of them and it should be considered before harvesting. Under Isparta or similar conditions, budding period and the beginning of flowering period are the best to getting to most harvest from alfalfa. Taking into account both results of yield and nutritive value of alfalfa based on Isparta's conditions, the best harvesting period was determined during the beginning of the flowering period.

Green herbage and forage yield of alfalfa: Green herbage and forage yields of alfalfa are presented in Table 1. Green herbage yield obtained from average states of maturity were affected by harvest factor ($P<0.05$). There is an interaction between harvest and maturity state if green herbage yield, obtained from harvests, is evaluated in terms of maturity state. Therefore, every maturity state was analyzed by itself. As can be seen from Table 1, the alfalfa hay's green herbage yield and crude protein yield were highest (9609.33 kg/da and 379.23 kg/da) in the budding period. Forage yield, dry matter yield, were highest (1791.89 kg/da, 1742.63 kg/da,) in the beginning of flowering period. The highest crude oil yield of alfalfa was determined in the full blooming period as 30.01kg/da. The highest and lowest values of total green herbage yield were determined in the budding period as 9609.33 kg/da and in the seed-setting period as 6948.83 kg/da, respectively. The green herbage yield of legumes is expected to increase during maturation because the plants have new organs and parts of the plants reach maximum levels. Some research supports an increase of herbage yield during maturation by Ayhan *et al*. (2004) (in some legume forage crops; white clover, birdsfoot trefoil, red clover, milkvetch, Peruvian alfalfa), Bakhashwain,(2010) (in rhodes grass-alfalfa) and Aksoy and Nursoy (2010) (in mixture of Hungarian vetch and wheat) but some researchers disagree (Seker, 2002) (in alfalfa). For the average forage yield, the lowest and highest values were determined (344.71 kg/da and 549.35 kg/da) in the budding and the seed setting period, respectively. The alfalfa total forage yield was highest (1791.89 kg/da) in the beginning of flowering period. Alfalfa forage yield was affected by harvest and maturity state, depending on increasing dry matter content ($P<0.05$). The results of experiment are in agreement with those reported results by Akyildiz (1986) (in legumes), Ayhan *et al*. (2004) (in some legume forage crops) and Aksoy and Nursoy (2010) (in mixture of Hungarian vetch and wheat). Average dry matter yield of alfalfa was highest (535.83 kg/da) in its most mature period of seed setting. Total dry matter yield of alfalfa was highest (1742.63 kg/da) in the beginning of the flowering period. Alfalfa dry matter yield were affected by maturity state ($P<0.05$). Maturity stage is an important factor affecting

TABLE 1: Green herbage and forage yield of alfalfa

Harvesting Periods				
Number of Harvest	Green Herbage Yield, kg/deciare			
	Budding period	Beginning of flowering period	Full blooming period	Seed setting period
1st	2726.00±119.89a	2880.67±52.20a	2450.33±390.61a	2263.50±191.80b
2nd	1808.33±112.51bc	1946.00±242.13b	1790.67±136.25bc	3335.33±203.19a
3rd	1616.67±251.66bc	1976.67±338.96b	2285.67±139.62ab	1350.00±100.00c
4th	1525.00±265.57c	1710.00±258.62b	1225.00±66.14c	-
5th	1933.33±57.74b	912.50±76.03c	-	-
Total GHY	9609.33	9425.84	7751.67	6948.83
Av. GHY	1921.87	1885.17	1937.92	2316.28
CV			P	
Harvest			.001	
		Forage Yield, kg/deciare		
1st	565.78±17.02a	588.41±22.77a	561.77±83.11a	635.73±45.00a
2nd	433.92±24.36ab	367.33±19.11b	415.75±31.25ab	567.47±37.11a
3rd	296.28±35.47bc	315.84±77.84b	469.64±46.31a	444.84±35.97b
4th	247.76±50.26c	360.03±40.27b	278.97±16.60b	-
5th	179.79±116.69c	160.28±8.78c	-	-
Total FY	1723.53	1791.89	1726.13	1648.04
Av. FY	344.71	358.38	431.50	549.35
CV			P	
Harvest			.004	
		Dry Matter Yield, kg/deciare		
1st	519.67±51.95a	575.62±19.98a	543.62±80.73a	621.16±43.87a
2nd	422.74±23.32ab	356.54±19.25b	402.30±30.29ab	547.99±34.88a
3rd	285.20±35.16bc	306.92±75.44b	450.93±48.83a	438.34±36.37b
4th	236.41±49.31c	349.61±42.10b	275.48±15.50b	-
5th	163.00±95.10c	153.94±8.74c	-	-
Total DMY	1627.02	1742.63	1672.33	1607.49
Av. DMY	325.40	348.53	418.08	535.83
CV			P	
Harvest			.005	
		Crude Protein Yield, kg/deciare		
1st	130.78±9.16a	112.36±6.05a	104.54±17.94a	100.52±8.41a
2nd	92.93±5.96b	69.00±3.93b	75.95±4.42ab	96.48±7.60a
3rd	64.52±8.63bc	68.49±17.24b	88.33±8.34a	75.44±4.96b
4th	53.36±9.15c	73.11±5.60b	54.95±3.74b	-
5th	37.64±23.89c	36.31±1.18c	-	-
Total CPY	379.23	359.27	323.77	272.44
Av. CPY	75.85	71.85	80.94	90.81
CV			P	
Harvest			.006	
		Crude Oil Yield, kg/deciare		
1st	8.18±0.50a	5.34±1.13a	12.01±1.91a	14.95±0.98a
2nd	5.34±0.20b	4.71±0.24a	5.79±0.39b	6.32±031b
3rd	3.34±0.41b	5.30±1.27a	7.37±1.72b	6.73±0.24b
4th	3.80±0.67b	2.44±0.40b	4.84±0.58b	-
5th	3.12±2.08b	2.44±0.26b	-	-
Total COY	23.78	20.23	30.01	28
Av. COY	4.76	4.05	7.50	9.33
CV	P		P	
Harvest	.001		.003	

The differences between the values in column with the same letters are statistically insignificant at $P\leq0.05$.

quality. In almost every plant, during the maturation while dry matter increases yield and stem ratio decreases in the leaf. The findings in this experiment are in agreement with those reports by Akyildiz (1986), Ayhan *et al*. (2004), Aksoy and Nursoy (2010). Average crude protein yield of Alfalfa was highest (90.81 kg/da) in the seed setting period but total crude protein yield was highest (379.23 kg/da) in the beginning of the flowering period. The highest crude protein yield was determined in the budding period but due to the high-level non-protein nitrogen content of the plant in this period care must be taken in terms of animal nutrition. Concerning the average crude oil yield, the lowest and highest values were determined in beginning of the flowering period as 4.05 kg/da and in the seed-setting period as 9.33 kg/da. The lowest and highest values of total crude oil yield were determined at the beginning of the flowering period as 20.23 kg/da and in the full flowering period as 30.01 kg/da. Crude oil yield was affected from harvest and maturity factors ($P<0.05$).

Chemical composition of alfalfa: The chemical composition of alfalfa (DM, OM, CP, EE, NFE, and Ash) harvested at different states of maturity is presented in Table 2. When averages of maturity stage evaluated DM and NFE were shown significant ($P<0.05$) differences in terms of harvests. As can be seen from Table 1, the alfalfa hay's average of DM and OM were highest (24.78 and 82.01%) in the seed setting period. Average of CP, NFE and Ash were highest (19.67, 32.28 and 11.21%) in the budding period. The highest average of EE was determined in the beginning of the flowering period as 1.57%. DM content increased with maturation if evaluation was made in terms of maturity ($P<0.05$). With increasing maturity, plants that have a high content of cellulose take the place of fresh plants that have high water content, and due to this DM content increases. The results of some researchers support our findings Avcioglu and Geren (1998) (in legume crops), Geren *et al*. (2003) (in vetch), Yu *et al*. (2003) (in timothy and alfalfa), Ayhan *et al*. (2004) (in some legume forage crops); Homolko *et al*. (2008) (in alfalfa), Aksoy and Nursoy (2010) (in mixture of Hungarian vetch and wheat) and Chatepa (2012) (in alfalfa). The lowest and highest values of average CP were determined in the seed-setting period as 15.26% and in the budding period as 19.67%, respectively. CP content decreased with maturation if evaluation was made in terms of maturity ($P<0.05$). Plants in the growing period synthesized more protein due to the area of photosynthesis surface, and they had higher protein content. These findings are in agreement with those reports by Madder *et al*. (1997); Avcioglu and Geren (1998), Yu *et al*. (2003), Cozzi *et al*. (2005), Dinic *et al*. (2005), Dolezal and Skladanka (2008), Homolko *et al*. (2008), Pop *et al*. (2010) in alfalfa. The highest ether extract value was determined at the beginning of the flowering period as 1.57%. At first, EE content increased, then it decreased with maturity in the second and third harvests ($P<0.05$). When averages of harvest were evaluated ash content decreased with maturity. Our findings support some researcher's results Manga (1978), Yolcu *et al*. (2000), Yu *et al*. (2003), Geren *et al*. (2003), Dinic *et al*. (2004), and Özyigit and Bilgen (2006) in legumes. The lowest value of average CS was determined in the budding period as 25.86%. CS content increased with maturation ($P<0.05$). The reason for low cellulose content is using of the synthesized assimilates to construct a new tissue at the beginning of growth period. Also, in later vegetation, the increase in stem ratio causes increased cellulose content. The findings in this experiment are in agreement with those reports by Homolko *et al*. (2008), Pop *et al*. (2010), Weir *et al*. (1960), Manga (1978), Yolcu *et al*. (2000) and Özyigit and Bilen (2006) in alfalfa. The lowest value of average NDF was determined in the budding period as 44.90%. NDF content increased with ($P<0.05$). Our findings support some researchers' results Yu *et al*. (2003), Cozzi *et al*. (2005), Aksoy and Nursoy (2010), Homolko *et al*. (2008), Dolezal and Skladanka (2008), Canbolat and Karaman (2009), Pop *et al*. (2010) in some legumes. NDF is composed of hemicellulose, cellulose and lignin. Although the hemicellulose content is less than the cellulose content, digestibility of hemicellulose is high but it decreases with maturation. NDF has an inverse relation with DMI and DDM and a positive relation with chewing time (Manga, 1978; Yolcu *et al*. 2000; Özyigit and Bilgen, 2006). The lowest value of average ADF was determined in the budding period as 32.78%. ADF content increased with maturation ($P<0.05$). The findings in this experiment are in agreement with those reported by Yu *et al*. (2003), Sayan *et al*. (2004), Dolezal and Skladanka (2008), Homolko *et al*. (2008), Canbolat and Karaman (2009), Aksoy and Nursoy(2010), Pop *et al*. (2010). ADF is composed of cellulose and lignin, and there is an inverse relationship between ADF and digestibility. The highest value of average ME was determined in the budding period as 1906.26 Kcal/kg. ME content decreased with maturation due to an increase in CS, ADF and NDF ($P<0.05$). Our findings support some researcher's reports Yu *et al*. (2003), Polat *et al*. (2007), Aksoy and Nursoy (2010) reported that hemicellulose, cellulose and lignin, which are important cell wall components of forage fiber, have a negative effect on digestibility and related with this on energy values. The highest value of average NEL was determined

TABLE 2: Chemical composition of Alfalfa (DM, OM, CP, EE, NFE, Ash)

Number of Harvest	Harvesting Period			
	Budding period	Beginning of flowering period	Full blooming period	Seed setting period
		Dry Matter (Dry matter basis, %)		
1st	21.21±0.28ab	22.31±0.31ab	24.12±0.26b	25.15±0.18b
2nd	21.86±0.17a	21.41±0.98b	26.56±0.32a	27.04±0.64a
3rd	21.08±0.34abc	21.82±0.62ab	22.93±0.49c	22.16±0.76c
4th	20.30±0.40c	23.63±1.17a	24.24±0.47b	-
5th	20.93±0.22bc	23.38±0.29a	-	-
Av. DM	**21.08**	**22.51**	**24.46**	**24.78**
CV	P	P		
Harvest	.002	.016		
		Organic Matter (Dry matter basis, %)		
1st	77.78±0.23b	79.98±0.21b	80.30±0.41a	84.92±0.19a
2nd	82.98±0.92a	82.03±0.52a	79.69±0.19ab	81.69±0.49b
3rd	78.62±0.44b	79.06±0.63b	78.33±0.38b	79.44±1.06c
4th	78.36±0.76b	79.32±0.25b	75.95±1.07c	-
5th	77.68±1.40b	75.83±0.60c	-	-
Av. OM	**79.08**	**79.24**	**78.57**	**82.01**
CV			P	
Harvest			.001	
		Crude Protein (Dry matter basis, %)		
1st	20.77±0.94a	17.23±0.55b	16.69±0.55a	14.85±0.54a
2nd	19.94±0.30ab	17.43±0.43b	16.67±0.37a	15.65±0.24a
3rd	19.62±0.46ab	19.37±0.29a	16.80±0.34a	15.29±0.30a
4th	19.33±0.59ab	18.00±0.17b	17.12±0.09a	-
5th	18.71±0.12b	19.45±0.41a	-	-
Av. CP	**19.67**	**18.30**	**16.82**	**15.26**
CV	P		P	P
Harvest	.029		.453	.103
		Ether Extract (Dry matter basis, %)		
1st	1.30±0.06b	2.57±0.17a	1.93±0.03a	2.19±0.05a
2nd	1.15±0.02c	1.19±0.05b	1.26±0.02b	1.03±0.02c
3rd	1.02±0.02d	1.44±0.06b	1.38±0.23b	1.37±0.07b
4th	1.38±0.03b	1.33±0.09b	1.52±0.08b	-
5th	1.53±0.06a	1.34±0.09b	-	-
Av. EE	**1.28**	**1.57**	**1.52**	**1.53**
CV			P	
Harvest			.004	
		Nitrogen Free Extract (Dry matter basis, %)		
1st	29.89±0.84b	30.62±0.47b	26.90±0.96b	30.63±1.54a
2nd	34.02±0.74a	34.67±1.09a	31.00±1.66a	28.86±0.97a
3rd	34.43±1.22a	29.11±0.72c	28.07±0.56b	28.80±1.50a
4th	30.05±1.05b	29.75±1.11bc	26.94±0.93b	-
5th	32.99±0.94a	27.80±0.54d	-	-
Av. NFE	**32.28**	**31.04**	**28.23**	**29.43**
CV	P			
Harvest	.001			
		Ash (Dry matter basis, %)		
1st	12.12±0.33a	10.62±0.11a	9.97±0.29b	8.18±0.17c
2nd	10.13±0.22c	10.98±0.30a	10.91±0.07b	10.40±0.20b
3rd	11.58±0.46ab	10.91±0.05a	10.23±0.27a	11.11±0.78a
4th	11.02±0.62bc	10.39±0.19a	11.76±0.15a	
5th	11.21±0.18ab	12.35±0.37a	-	
Av. Ash	**11.21**	**11.05**	**10.72**	**9.90**
CV			P	
Harvest			.004	

The differences between the values in column with the same letters are statistically insignificant at P≤0.0

in the budding period as 1396.72 Kcal/kg. NEL content decreased with maturation in the first and third harvests (P<0.05). Aksoy and Nursoy (2010) reported that NEL content increased with maturity.

Dynamics of cell walls content and energy values of alfalfa: Dynamics of cell walls content and energy values of alfalfa harvested at different states of maturity are presented in Table 3. As can be seen from Table 3, the alfalfa hay's CS, NDF and ADF were lowest (25.86%, 44.90% and 32.78%) and its ME and NEL were highest (1906.26 Kcal/kg and 1396.72 Kcal/kg) in the budding period. CS, NDF and ADF content increased with maturation (P<0.05). The reason for low cellulose content is using of the synthesized assimilates to construct a new tissue at the beginning of

TABLE 3: Dynamics of cell wall content and energy values of alfalfa

Number of Harvest	Harvesting Period			
	Budding period	Beginning of flowering period	Full blooming period	Seed setting period
		Crude Cellulose (Dry matter basis, %)		
1st	25.83±0.31[b]	29.56±1.23[ab]	34.79±0.91[a]	37.25±1.16[a]
2nd	27.88±0.11[a]	28.74±0.79[b]	30.76±1.44[c]	36.15±0.74[a]
3rd	23.55±0.70[c]	29.14±0.60[b]	32.07±0.70[b]	33.97±0.60[b]
4th	27.61±0.87[a]	30.25±0.95[a]	30.37±0.82[c]	-
5th	24.45±0.32[c]	27.24±0.22[c]	-	-
Av. CC	**25.86**	**28.99**	**32.00**	**35.79**
CV		P		
Harvest		.001		
		NDF (Dry matter basis, %)		
1st	40.98±0.41[b]	53.15±1.68[a]	52.48±1.45[a]	57.49±2.65[a]
2nd	48.25±0.36[a]	45.53±3.20[b]	45.15±2.14[b]	52.52±3.53[ab]
3rd	41.75±1.87[b]	45.51±3.83[b]	52.91±1.79[a]	51.52±1.90[b]
4th	48.29±1.81[a]	48.30±3.33[ab]	51.72±1.40[a]	-
5th	45.24±3.74[ab]	44.66±2.25[b]	-	-
Av. NDF	**44.90**	**47.43**	**50.57**	**53.84**
CV	P	P	P	P
Harvest	.007	.021	.008	.009
		ADF (Dry matter basis, %)		
1st	30.81±0.60[ab]	40.22±1.52[a]	39.82±1.53[a]	43.39±2.78[a]
2nd	36.05±1.06[a]	34.60±2.96[abc]	32.46±3.49[b]	39.60±2.17[ab]
3rd	28.10±3.44[b]	33.45±3.55[bc]	40.62±1.56[a]	37.90±0.72[b]
4th	35.69±2.81[a]	37.54±2.41[ab]	37.55±0.87[ab]	-
5th	33.24±1.55[ab]	29.40±1.13[c]	-	-
Av. ADF	**32.78**	**35.04**	**37.61**	**40.30**
CV	P	P	P	P
Harvest	.008	.005	.018	.005
		Metabolisable Energy, K.cal/kg DM		
1st	1888.10±0.05[b]	1794.89±0.27[b]	1527.21±0.21[c]	1546.33±0.20[a]
2nd	1921.56±0.15[b]	1854.64±0.22[a]	1680.17±0.25[a]	1479.41±0.17[a]
3rd	1993.26±0.13[a]	1754.26±0.19[b]	1565.45±0.11[bc]	1522.43±0.21[a]
4th	1799.67±0.14[c]	1689.73±0.19[c]	1596.52±0.17[ab]	
5th	1928.73±0.17[b]	1754.26±0.12[b]		
Av. ME	**1906.26**	**1769.56**	**1592.34**	**1516.06**
CV	P	P	P	P
Harvest	0.001	0.001	0.052	0.002
		Net Energy Lactation, K.cal/kg DM		
1st	1398.15±0.34[b]	1300.16±0.14[cd]	1161.54±0.10[a]	1097.01±0.13[b]
2nd	1343.18±0.01[c]	1321.67±0.09[b]	1269.09±0.16[a]	1125.69±0.08[b]
3rd	1457.90±0.08[a]	1312.11±0.07[bc]	1233.24±0.08[a]	1183.05±0.66[a]
4th	1350.35±0.10[c]	1281.04±0.10[d]	1278.65±0.02[a]	
5th	1434.00±0.04[a]	1359.91±0.02[a]		
Av. NEL	**1396.72**	**1314.98**	**1235.63**	**1135.25**
CV		P	P	
Harvest		.001	.008	

The differences between the values in column with the same letters are statistically insignificant at P≤0.05

growth period. Also, in later vegetation, the increase in stem ratio causes increased cellulose content. The findings in this experiment are in agreement with those reported by Weir *et al*. (1960), Manga (1978), Yolcu *et al*. (2000), Yu *et al*. (2003), Sayan *et al*. (2004), Cozzi *et al*. (2005), Özyigit and Bilen (2006), Dolezal and Skladanka (2008) Homolko *et al*. (2008), Canbolat and Karaman (2009), Aksoy and Nursoy (2010), Pop *et al*. (2010) in some legumes. NDF is composed of hemicellulose, cellulose and lignin. Although the hemicellulose content is less than the cellulose content, digestibility of hemicellulose is high but it decreases with maturation. NDF has an inverse relation with DMI and DDM and a positive relation with chewing time (Manga, 1978; Ozyigit and Bilgen, 2006) in alfalfa. ADF is composed of cellulose and lignin, and there is an inverse relationship between ADF and digestibility. The lowest and highest values of average ME were determined in the seed-setting period as 1516.06 K.cal/kg and in the budding period as 1906.26 K.cal/kg. ME content decreased with maturation due to an increase in CS, ADF and NDF if evaluation is made in terms of maturity ($P<0.05$). Our findings support some researcher's reports Yu *et al*. (2003), Polat *et al*. (2007), Aksoy and Nursoy (2010) reported that hemicellulose, cellulose and lignin, which are important cell wall components of forage fiber, have a negative effect on digestibility and related with this on energy values. The lowest and highest values of average NEL were determined in the seed-setting period as 1135.25 K.cal/kg and in the budding period as 1396.72 K.cal/kg. NEL content decreased with maturation in the first and third harvests ($P<0.05$). Aksoy and Nursoy, (2010) reported that NEL content increased with maturity.

Quality of forages values of alfalfa: The quality of forage values of alfalfa harvested at different states of maturity is presented in Table 4. As can be seen from Table 4, the alfalfa hay's average of DDM, TDN, DMI and RFV were highest (63,37%, 57,75% 2,69%, 132) in its most fresh period of budding. DDM content was increased by maturation ($P<0.05$), and there is an inverse relationship between DDM and ADF. The findings in this experiment are in agreement with those reports by Marten *et al*. (1988) (in alfalfa), Yu *et al*. (2003) (in timothy and alfalfa), Redfearn and Zhang (2004) and Canbolat and Karaman (2009) in legumes, Aksoy and Nursoy (2010) (in vetch and wheat mixture). The highest value of average TDN was determined in the budding period as 57.75%. TDN content decreased with maturation ($P<0.05$), and there is an inverse relationship between TDN and ADF. These findings are in accordance with some researcher's reports Aksoy and Nursoy (2010) (in vetch and wheat mixture), Yu *et al*. (2003) (in timothy and alfalfa). The highest value of average DMI was determined in the budding period as 2.69%. DMI content decreased with maturation ($P<0.05$). There is a negative relationship between DMI and NDF. These findings are in agreement with those reports by Aksoy and Nursoy (2010) (in vetch and wheat mixture), Canbolat and Karaman (2009) (in legumes). In grass, TDN, DDM and DMI values, which are directly related with the ADF and NDF content, are expected to be high, because this generally fresh plant has a lower lignin content at the beginning of the vegetation period (Cakmakci *et al*., 2005) (in vetch and perennial ryegrass). The highest value of average RFV was determined in the seed-setting period as 100 and in the budding period as 132. The RFV index estimates the DDM of the alfalfa from ADF, and calculates the DM intake potential (as a percent of body weight, BW) from the NDF. The index is then calculated as DDM multiplied by dry matter intake (DMI as a % of BW) and divided by 1.29. RFV decreased with maturation ($P<0.05$). The index ranks forages relative to the digestible DMI of full bloom alfalfa, assuming 41% ADF and 53% NDF. The RFV index is 100 at this growth stage. According to this classification, best quality forages may be obtained from all maturity stages except the seed-setting period. Dunham (1998) reported that alfalfa RFV is 164 before the budding period and decreases with maturity. Canbolat *et al*. (2006) determined the RFV at the beginning of the flowering period as 145. Our findings are in accord with some researcher's reports such as Canbolat and Karaman (2009) (in alfalfa), Aksoy and Nursoy (2010) (mixture of Hungarian vetch and wheat).

Macro and micro minerals content of alfalfa: Macro and micro mineral matter content of alfalfa, as influenced by maturity state, are shown in Table 5. As can be seen from Table 5, the alfalfa's average of P and Zn were highest (0.25% and 16.32 ppm) in the budding period. The highest average of Ca, Cu and Mn were determined (1.08%, 17.13% and 57.26 ppm) in the seed setting, full blooming and beginning of flowering periods, respectively. Na content of alfalfa was determined as 0.04% and was not affected by harvest or maturity stage ($P>0.05$). The lowest values of average P and Ca were determined in the seed setting period as 0.21% and at the beginning of flowering as 0.88%, and the highest values of average P and Ca were determined in the budding period as 0.25% and in the seed setting period as 1.08%. Na content of alfalfa was determined as 0.04% and was not affected by harvest or maturity stage ($P>0.05$). The optimum Na content of a forage crop is at least 0.2%. P content was affected by different harvest time. Vaneys and Reid (1987) and Yolcu *et al*. (2000), reported in studies with different plants that the

TABLE 4: Quality of forages values of alfalfa

Number of Harvest	Harvesting Period			
	Budding period	Beginning of flowering period	Full blooming period	Seed setting period
		Digestibility Dry Matter, %		
1st	64.90±0.47[ab]	57.57±1.17[c]	57.88±1.19[b]	55.10±2.16[b]
2nd	60.82±0.87[b]	61.95±2.31[abc]	63.61±2.71[a]	58.05±1.68[ab]
3rd	67.01±2.69[a]	62.84±2.77[ab]	57.26±1.21[b]	59.38±0.56[a]
4th	61.10±2.19[b]	59.66±1.88[bc]	59.65±0.68a[b]	-
5th	63.01±1.21[ab]	66.00±0.88[a]	-	-
Av. DDM	**63.37**	**61.40**	**59.60**	**57.51**
CV	P	P	P	P
Harvest	.008	.005	.018	.005
		Total Digestible Nutrients, %		
1st	59.23±0.45[ab]	52.15±1.13[c]	52.46±1.15[b]	49.77±2.09[b]
2nd	55.29±0.79[b]	56.38±2.22[abc]	57.99±2.62[a]	52.61±1.62[ab]
3rd	61.26±2.59[a]	57.24±2.67[ab]	51.85±1.17[b]	53.90±0.54[a]
4th	55.56±2.11[b]	54.17±1.81[bc]	54.16±0.66[ab]	-
5th	57.40±1.17[ab]	60.29±0.85[a]	-	-
Av. TDN	**57.75**	**56.05**	**54.12**	**52.09**
CV	P	P	P	P
Harvest	.008	.005	.018	.005
		Dry Matter Intake, BW %		
1st	2.93±0.03[a]	2.26±0.07[b]	2.29±0.07[b]	2.09±1.00[a]
2nd	2.49±0.02[b]	2.65±0.20[ab]	2.66±0.13[a]	2.29±0.20[a]
3rd	2.88±0.13[a]	2.65±0.23[ab]	2.27±0.08[b]	2.33±0.09[a]
4th	2.49±0.09[b]	2.49±0.17[ab]	2.32±0.06[b]	-
5th	2.67±023[ab]	2.69±0.14[a]	-	-
Av. DMI	**2.69**	**2.55**	**2.39**	**2.24**
CV	P	P	P	P
Harvest	.006	.036	.009	.029
		Relative Feed Value		
1st	147±2.49[a]	101±5.16[b]	103±4.36[b]	89±7.49[a]
2nd	117±2.40[b]	127±14.10[ab]	131±12.11[a]	103±9.74[a]
3rd	149±2.66[a]	129±16.47[ab]	101±5.54[b]	107±4.93[a]
4th	118±8.47[b]	115±11.11[ab]	107±4.06[b]	-
5th	130±13.64[ab]	138±7.97[a]	-	-
Av. RFV	**132**	**122**	**111**	**100**
CV	P	P	P	P
Harvest	.003	.019	.011	.012

The differences between the values in column with the same letters are statistically insignificant at $P \leq 0.003$.
BW= Body weight
Calculations of DDM, TDN, DMI and RFV are given in materials and methods.

P content of alfalfa decreased with maturity. At the beginning of the vegetation period, plants have a high mineral matter content because of their high water content in the same period (Aydemir and Ince, 1988). Ca content of alfalfa was not affected by harvest factor. The plant, owing to high air and soil moisture in spring, prevents transportation of Ca. This causes a low proportion of Ca in this period. Cu and Mn were not affected by harvest or maturity stage ($P>0.05$). The highest values of average Cu and Mn were in the full flowering period (17.13%) and at the beginning of flowering (57.26%). Cu has an effective role on nodule generation in forage crops and nodulation decreases with Cu defiance and is determined the less N fixation (Marschner, 1995) (in alfalfa). Mn is important for the control of water use. If there is a deficiency of Mn it causes latency in flowering (Kacar and Katkat, 1998) (in alfalfa). Zn content decreased with maturation if evaluation is made in terms of maturity stages ($P<0.05$). Alfalfa benefits the most from Zn compared to other plants, and Zn has an affirmative effect on flowering and nodulation (Kacar and Katkat, 1998).

TABLE 5: Macro and micro minerals content of alfalfa

Number of Harvest	Harvesting Period			
	Budding period	Beginning of flowering period	Full blooming period	Seed setting period
		P, %		
1st	0.22±0.03[c]	0.21±0.00[c]	0.20±0.00[d]	0.19±0.00[c]
2nd	0.19±0.01[d]	0.24±0.02[a]	0.21±0.01[c]	0.23±0.03[a]
3rd	0.31±0.03[a]	0.25±0.01[a]	0.23±0.02[b]	0.20±0.01[b]
4th	0.26±0.01[b]	0.22±0.01[b]	0.24±0.03[a]	-
5th	0.25±0.02[b]	0.22±0.02[b]	-	-
Av. P	**0.25**	**0.23**	**0.22**	**0.21**
CV	P	P	P	P
Harvest	.001	.061	.117	.000
		Ca, %		
1st	0.94±0.13[a]	0.89±0.03[a]	1.19±0.16[ab]	1.43±0.12[a]
2nd	1.03±0.13[a]	0.69±0.11[a]	0.78±0.16[b]	0.73±0.12[c]
3rd	1.18±0.07[a]	0.95±0.16[a]	0.85±0.06[b]	1.07±0.05[b]
4th	1.02±0.31[a]	0.93±0.19[a]	1.32±0.13[a]	-
5th	0.96±0.04[a]	0.95±0.07[a]	-	-
Av. Ca	**1.03**	**0.88**	**1.04**	**1.08**
CV	P	P	P	P
Harvest	.263	.337	.007	.000
		Na, %		
1st	0.04±0.00[a]	0.04±0.00[a]	0.04±0.00[a]	0.04±0.00[a]
2nd	0.03±0.01[a]	0.04±0.00[a]	0.03±0.01[a]	0.04±0.00[a]
3rd	0.03±0.00[a]	0.04±0.00[a]	0.04±0.00[a]	0.04±0.00[a]
4th	0.03±0.00[a]	0.04±0.00[a]	0.04±0.00[a]	-
5th	0.05±0.01[a]	0.05±0.00[a]	-	-
Av. Na	**0.04**	**0.04**	**0.04**	**0.04**
CV	P	P	P	P
Harvest	.028	.008	.092	.197
		Cu, ppm		
1st	18.88±0.64[a]	19.86±3.74[a]	20.95±1.97[a]	18.77±1.02[a]
2nd	12.92±3.82[a]	16.74±0.68[a]	14.21±2.94[a]	15.81±1.53[a]
3rd	18.16±3.24[a]	16.32±2.66[a]	15.43±4.87[a]	12.47±0.77[a]
4th	13.91±1.39[a]	18.87±4.71[a]	17.93±7.79[a]	-
5th	14.14±0.35[a]	13.81±0.32[a]	-	-
Av. Cu	**15.60**	**17.12**	**17.13**	**15.68**
CV	P	P	P	P
Harvest	.001	.269	.374	.003
		Mn, ppm		
1st	53.00±5.47[ab]	55.04±5.75[b]	51.95±4.30[a]	44.93±0.00[a]
2nd	43.65±3.84[b]	48,58±5,33[b]	48,54±7,78[a]	54,81±0,00[a]
3rd	58,22±3,27[a]	57,07±6,29[b]	50,02±4,23[a]	53,25±0,00[a]
4th	56,03±2,54[a]	52,74±3,06[b]	63,51±4,94[a]	-
5th	56,55±1,36[a]	72,85±1,06[b]	-	-
Av. Mn	**53,49**	**57,26**	**53,51**	**51,00**
CV	P	P	P	P
Harvest	.013	.003	.067	.046
		Zn, ppm		
1st	15.72±0.46[b]	14.51±1.23[ab]	13.40±0.26[a]	13.81±0.81[ab]
2nd	13.13±2.07[b]	18.09±2.02[a]	15.45±1.53[a]	16.13±1.19[ba]
3rd	22.46±3.07[a]	18.36±2.50[a]	14.07±0.57[a]	11.56±0.50[b]
4th	15.30±0.71[b]	13.15±0.52[b]	15.33±0.40[a]	-
5th	14.99±0.72[b]	15.70±0.35[ab]	-	-
Av. Zn	**16.32**	**15.96**	**14.56**	**13.83**
CV	P	P	P	P
Harvest	.001	.021	.042	.009

The differences between the values in column with the same letters are statistically insignificant at $P \leq 0.05$.

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