

Harvest management and cultivar effect on alfalfa (*Medicago sativa* L.) forage quality

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Abstract

Understanding the connection between alfalfa (*Medicago sativa* L.) harvesting stage and forage quality will allow optimum timing of harvest. A field experiment was established to study the variation of forage quality of six cultivars of alfalfa harvested at three stages of harvest duration. The forage quality of alfalfa cultivars was significantly different at different stages of plant development. Crude fiber, NDF and ADF increased with increasing plant age ranging from 16.9%, 40.27 g kg⁻¹ DM and 23.3 g kg⁻¹ DM to 26.4%, 49.38 g kg⁻¹ DM and 41.33 g kg⁻¹ DM, respectively; while leaf to stem ratio, crude protein, DMD, TDN, DMI, RFV and ME decreased from 1.54%, 72.33 g kg⁻¹ DM, 71.25 g kg⁻¹ DM, 3.01 g kg⁻¹ DM, 175.33% and 1.29 MJ to 1.12%, 50.66 g kg⁻¹ DM, 48.03 g kg⁻¹ DM, 2.45 g kg⁻¹ DM, 85.25% and 6.61 MJ. Difference in the forage quality among alfalfa cultivars can be mainly caused by genetic diversity of plants and variation in the partitioning of assimilates to reproductive parts. The recognized superior cultivar will be used to improve the nutritional value of forage crops in the breeding programs. Our data suggests that the relationship between forage quality and harvest time had significant effect on forage quality than cultivar differences or genetic selection.

Highlights

- In arid and semi-arid regions, the cultivars Fasta and Meldor meet all forage quality standards.
- Early harvesting may be an effective strategy to enhance forage quality.
- Lower level of protein might be the reason of loss of leaves and higher rate of stem in total canopy.

Introduction

Alfalfa (*Medicago sativa* L.) is a forage legume with high quality and yield which requires lower nitrogen fertilizer than other forage crops due to its ability to fix atmospheric nitrogen. Considering the rising prices of maize due to its higher irrigation requirements and use in biofuel industry, alfalfa hay might be a promising alternative for forage and hay production [1]. Balancing quality and quantity of alfalfa depends more on harvesting stage than on genetic selection. Of course, genetic factors should also be considered but the choice of the best cultivar and stage of cutting should be considered together to achieve the highest yield with high nutritive value. This means that changes in harvesting time affect the ranking of cultivars in terms of forage quality [2]. Genetic improvement of alfalfa by routine plant breeding has been focusing on traits such as forage yield and forage nutritive value in arid and semi-arid regions. However, selection of suitable cultivar for arid and semi-arid regions is one of the strategic management approaches to justify the production of plants per unit area. Harvest schedule is another important management strategy that can increase the quality and quantity of plants. It has been reported that the best harvesting time for alfalfa is associated with flowering time [3, 4]. Although early harvesting guarantees a higher protein content but the early cut strategy is not suitable due to lower yield production. The stems make up more than 50% of dry matter in alfalfa and as the stems mature, the fiber concentration enhances [5]. Therefore, harvesting time can change the digestibility of alfalfa which impacts the dairy market substantially by reducing animal waste, increased animal productivity and reduced feed costs.

In summary, early harvest leads to high protein and DMD values but yielded low hay while, late cut resulted in high hay yield with low protein and DMD values. Each producer aims to obtain forage with higher nutritive value with minimum reduction in biomass, but the challenge is determining how much the improvement in protein and nutritive value of forage balances the loss of herbage yield. Also, in arid and semi-arid regions with limited water availability, the selection of most suitable cultivar must be considered. Therefore, the objectives of the current study were: 1) evaluation of LAI and leaf to stem ratio, 2) investigation of genetic variation in forage quality and 3) determine of best alfalfa cultivars.

Materials and Methods

This experiment was conducted on a 2-year old stands of six alfalfa (*Medicago sativa* L.) cultivars at the research farm of Islamic Azad University (Varamin, 918 m.a.s.l, 35°32'52" N, 51°64'72"E) on silty loam soil. Plots were assigned in a randomized complete block design with a factorial arrangement of six cultivars (Fasta, Timbale, Melissa, Hamadani, Meldor, and Sovrana) and three first cut management schedules (at 10, 50 and 100% flowering stage) with three replications. Soil analyses showed that the levels of nitrogen, phosphorous and potassium were 0.17%, 5.1 mg kg⁻¹ and 300 mg kg⁻¹ with soil pH between 7.4 and 7.8. Monthly temperature and precipitation data were recorded each year (Fig. 1). Phosphorous and potassium were amended each fall to meet recommendations for alfalfa production. The plots were 1×5 m and consisted of 5 rows spaced 20 cm apart. The seeds (20 kg ha⁻¹) of alfalfa cultivars were drilled (20-cm row spacing) in the first week of February 2017, which was the year of establishment. In the first two years of plant life (2017 and 2018), all cultivation practices were carried out based on local recommendations, and all plants were cut twice in order to ensure successful plant establishment [6, 7].

Harvest treatments were laid out in 2019 and 2020 (late June-late August), between 09:00 am and 11:00 am when 10, 50 and 100% flowers had appeared. Leaf area index was measured using an electronic leaf area meter, LI-3100-LICOR, Lincoln, NE, USA. Estimation of total dry matter was done by harvesting 1 m² of herbage from the center of each plot. The cuts were initiated 5 cm above the soil surface. In each cut, a 100 g sample of fresh herbage was weighed, separated in two plant components (leaf and stem) and dried at 60 °C for 72 hours, after which the leaf to stem ratio was calculated.

For herbage analytical determination (crude fiber, NDF and ADF), dried samples were ground to 1 mm particles and analysed by near-infrared reflectance spectroscopy. Crude protein content was calculated as nitrogen $\times$ 6.25 [8]. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) content was determined by the Filter Bag Technique [9]. Dry matter digestibility (DMD) was estimated according to the following Eq. (1), in which all values are given g kg^{-1} on a dry matter basis [10]:

DMD = 88.9 – (0.779 $\times$ ADF)
Equation 1

The total digestible nutrients (TDN), dry matter intake (DMI), relative feed value (RFV), metabolism energy (ME) and net energy for lactation (NEL) values were determined using the following formulas [11]:

TDN = – 1.291 $\times$ ADF
Equation 2
DMI = 120 / NDF
Equation 3
RFV = DMD $\times$ DMI $\times$ 0.775
Equation 4
ME = (0.17 $\times$ DMD)-2
Equation 5
NEL= (1.044-(0.0119 $\times$ ADF) $\times$ 2.205
Equation 6

Analysis of variance (ANOVA) was used to estimate the interaction between year, cultivar and harvesting stage and LSD test was conducted for comparison of mean values at 5% probability level. SAS 9.1 and S-PLUS ver. 6.1 software, USA was used for Principal Component Analysis (PCA) based on biplot analysis [12].

Results

Analysis of variance

The results showed that year, harvesting stage, and cultivar treatments had a significant effect on all traits. Year had significant influence on leaf/stem ratio, leaf area index, crude fiber, ADF, TDN and NEL. The analysis of variance (Table 1) indicated that both harvesting stages and cultivar treatments had significant effect on all studied traits. The three way interaction between year, harvesting stage and cultivar had a significant effect on traits including leaf/stem ratio, leaf area index, crude fiber, ADF, TDN, RFV and NEL. Significant differences between cultivars were observed over the two years for leaf/stem ratio, leaf area index, crude fiber, NDF, DMD, DMI, RFV and ME. There was a significant interaction between year and harvesting stage for leaf/stem ratio, leaf area index, crude fiber, NDF, ADF, TDN, DMI, RFV and ME. Except crude protein, all studied traits were significantly affected by the interaction between harvesting stage $\times$ cultivar treatments.

Cluster and PCA analysis

The results of PCA (Figure 2B) showed that the two components (first and second) had an Eigen value greater than one and were selected as the most effective components accounting for 98.45% of the total variance. The biplot analysis revealed that 10% flowering+ Fasta, 10% flowering+ Melissa, 10% flowering+ Sovrana, 50% flowering+ Melissa and 50% flowering+ Hamadani placed in the same group due to their high affinity which associated with NDF. In addition, 10% flowering+ Meldor, 100% flowering+ Timbale, 100% flowering+ Meldor and 100% flowering+ Sovrana clustered together in the same group and showed a strong correlation with crude protein, ADF, DMD, TDN, ME and NEL.

The dendrogram obtained from the cluster analysis (Figure 2A) showed that the treatments in the present experiment were divided into three distinct groups based on all the measured traits. In the first group, five treatments 100% flowering+ Fasta, 100% flowering+ Hamadani, 50% flowering+ Meldor, 100% flowering+ Melissa and 10% flowering+ Fasta were placed in one cluster due to their high affinity. In the second group, the largest number of treatments (8 treatments) were clustered together based on proximity, which included 100% flowering+ Sovrana, 100% flowering+ Timbale, 10% flowering+ Meldor, 50% flowering+ Melissa, 50% flowering+ Hamadani, 10% flowering+ Sovrana, 100% flowering+ Meldor and 10% flowering+ Melissa. Cluster analysis separated five treatments- 10% flowering+ Hamadani, 10% flowering+ Timbale, 50% flowering+ Fasta, 50% flowering+ Timbale and 50% flowering+ Sovrana due to high relatedness in the third cluster.

Leaf/stem ratio

Averaged by harvesting stage and cultivar, all plants in 2020 had a higher leaf/stem ratio compared to 2019 (Table 2). In 2019, the maximum leaf/stem ratio was observed in cultivars Fasta, Hamadani and Meldor at 10% flowering stage (1.54) followed by Sovrana cultivar harvested at 50% flowering stage (1.32) and Meldor cultivar at 100% flowering stage (1.31) which were statistically at par. However, the leaf/stem ratio of Timbale cultivar was not affected by harvesting stage. In 2020, the highest leaf/stem ratio was recorded in Fasta cultivar at 10% flowering stage (1.76) followed by Hamadani cultivar at 10% flowering stage and Meldor at 50% flowering stage (1.48). At the 10% flowering stage, no significant difference was observed among Meldor, Melissa, Sovrana and Timbale cultivars

Leaf area index

The leaf area index of alfalfa cultivars was significantly affected by three-way interaction of year, harvesting stage and cultivar (Table 2). In 2019, Fasta cultivar harvested at 10% flowering stage yielded 43.3% and 43.8% higher leaf area index compared to 50% and 100% flowering harvesting stages, respectively. Averaged across all cultivars in 2019, plants harvested at 10% flowering stage had 29.02% and 31.2% higher leaf area index than those harvested at 50% and 100% flowering stages, respectively. Likewise, at two levels of harvesting stage (50% and 100% flowering stage) almost all cultivars were in the same statistical level and there was no significant different among them. In 2020, Fasta cultivar harvested at 10% flowering stage (4.24) and recorded significantly higher leaf area index than any other cultivars. However, different results were obtained at mid and late flowering stage (50% and 100% flowering stage). Alfalfa cultivars of Timbale (3.22), Sovrana (3.18) and Melissa (3.03) at 50% flowering stage and alfalfa cultivars Fasta (2.31) and Meldor (2.28) at 100% flowering stages showed significantly higher leaf area index than other cultivars.

Crude protein

A high variability in crude protein was found among the harvesting stage and cultivar (Table 3). The average value of crude protein was higher in samples harvested at the 10% flowering stage which was higher than 50% and 100% flowering stage by 5.7% and 10.8%, respectively. Data showed that cultivar of Hamadani had maximum crude protein (17.52%) followed by Melissa (17.31%), Meldor (17.22%) and Timbale (17.18%) while minimum crude protein (16.55%) was observed in Sovrana cultivar.

Crude fiber

The mean comparison of year× harvesting stage× cultivar interaction (Table 2) revealed differences between two years. In 2019, the highest crude fiber was obtained at 100% flowering stage in alfalfa cultivars of Fasta (23.10%), Hamadani (23.10%) and Meldor (21.80%). However, diverging results were observed in 2020, when the maximum crude fiber was obtained in alfalfa cultivar of Fasta (26.40%) at 100% flowering stage (Table 3). Averaged across harvesting stages, the Hamadani cultivar had the highest crude fiber content in both 2019 and 2020. Harvesting at 100% flowering stage caused increased in crude fiber and in both years of 2019 and 2020 (on average across cultivars), the highest crude fiber was obtained in plants harvested at 100% flowering stage.

Neutral detergent fiber

The mean comparison (Table 4) indicated that NDF value decreased with advancing plant maturity stage (In both years). Accordingly, harvesting at 100% flowering stage was higher than 10% flowering and 50% flowering by 13.59% and 13.95% in 2019 and 18.44% and 9.11% in 2020, respectively (Table 4). Average over harvesting stages, NDF of alfalfa forage in 2020 was slightly lower than 2019 (some data not shown). In addition, when the NDF content was examined in two years, it seems that NDF content varies from 37 (g kg⁻¹ DM) to 48.88 (g kg⁻¹ DM) and 39.33 (g kg⁻¹ DM) to 50.11 (g kg⁻¹ DM) in 2019 and 2020, respectively (Table 5). Fasta (in 2020) and Meldor (in 2019 and 2020) were found superior to the other cultivars. Notably, averaged across years, Meldor and Melissa recorded maximum and minimum content of NDF (Table 5). At 50% and 100% flowering stage, Meldor and Timbale exhibited the maximum of NDF content (Table 6). These attributes became almost stable at 10% flowering stage and the cultivar of Meldor had the highest NDF content followed by Timbale cultivar. While the lowest NDF content was recorded in Fasta and Melissa cultivar which were statistically similar. Averaged across cultivars, harvesting at 100% flowering stage led to the highest NDF content.

Acid detergent fiber

The interaction between harvesting stage and cultivar in 2019 revealed that the highest ADF content was recorded in the Meldor cultivar (40.66 g/kg DM) at the 100% flowering stage, followed by Timbale, which was in the same statistical group (Table 2). However, averaged across harvesting stage, Timbale had the highest ADF in 2019. This attribute had almost similar trend for all cultivars and in 2019 the content of ADF increased gradually by mortality of plants but in 2020 in the advanced stage of development, ADF increased and then decreased at the last harvest. In this regard, Fasta had lowest ADF content in all levels of harvesting stage but Meldor (41.33 g kg⁻¹ DM) and Timbale (40.33 g kg⁻¹ DM) harvested at 50% flowering and Timbale harvested at 100% flowering stage (40.33 g kg⁻¹ DM) registered the highest value ADF content. Averaging across the harvesting stages, the cultivars of Fasta, Hamadani, Meldor, Melissa, Savrana and Timbale had higher ADF content in 2020 rathan 2019 by average of 5.63, 9.33, 9.23, 11.92, 1.94 and 7.8%, respectively (Table 2).

Dry matter digestibility

DMD content varied significantly among cultivars and the trends were relatively consistent over both the years (Table 5). In 2019, Fasta (64.55 g kg⁻¹ DM) and Melissa (62.77 g kg⁻¹ DM) recorded highest DMD content in alfalfa forage while the cultivar of Timbale (52.88 g kg⁻¹ DM) recorded

significantly lower DMD content. In, 2020 the DMD content ranged from 51.0 g kg⁻¹ DM to 66.44 g kg⁻¹ DM with Fasta recording highest and Timbale the lowest DMD content. Forage DMD content of all cultivars varied significantly across harvesting stage (Table 6). Considering the effect of harvesting stage × cultivar, the results showed that the DMD content of all cultivars varied in each level of harvesting stage. Although, the cultivar of Fasta found suitable to be harvested at 10% flowering stage, but at 50% and 100% flowering stage, the cultivars of Melissa and Fasta exhibited highest DMD content. On average across cultivars, DMD content declined, with the advancement of growth stage, from 10% to 100% flowering stage.

Total digestible nutrients

The overall analysis showed that the TDN of forage quality of all cultivars dramatically decreased by mortality in both years (Table 2). In 2019, the highest value of TDN, was observed in cultivar of Fasta harvested at 10% and 50% flowering stage (71.25 and 70.82 g kg⁻¹ DM, respectively). On average of harvesting stage, Fasta and Melissa were found superior to the other cultivars (some data not shown). In 2020, the content of TDN in different alfalfa cultivars ranged from 53.19 (g kg⁻¹ DM) to 70.39 (g kg⁻¹ DM) at 10% flowering stage, 49.32 (g kg⁻¹ DM) to 63.94 (g kg⁻¹ DM) at 50% flowering and 49.32 (g kg⁻¹ DM) to 62.65 (g kg⁻¹ DM) at 100% flowering stage. The averaged TDN contents across all cultivars were 61.21 (g kg⁻¹ DM) at 10% flowering stage, but in the advanced stage of development (50% flowering stage) TDN decreased (54.98 g kg⁻¹ DM) and then increased at 100% flowering stage (56.48 g kg⁻¹ DM). In 2020, the highest TDN content was recorded in Fasta at 10% flowering stage. However, compared to the first harvesting stage, the content of TDN in Fasta declined by 9.16% and 10.9% at the second and third harvesting stages.

Dry matter intake

The evaluation of DMI showed significant variations among cultivars and harvesting stages over two years as well as in the combined harvesting stage × cultivar. In 2019, the highest content of DMI (Table 4) was observed at harvesting stage of 10% and 50% flowering. In 2020, plants harvested at 10% flowering ranked first in terms of DMI (3.01 g kg⁻¹ DM) which was significantly higher than 50% and 100% flowering by 10.63% and 18.6%, respectively. However, averaging across the harvesting stages, the DMI content was higher in 2019 than in 2020. Regarding year × cultivar interaction (Table 5), alfalfa cultivars Melissa and Meldor exhibited highest (3.28 g kg⁻¹ DM) and lowest (2.46 g kg⁻¹ DM) DMI compared to other cultivars in 2019. Likewise, in 2020, the DMI content ranged from 3.07 g kg⁻¹ DM (Fasta cultivar) to 2.41 g kg⁻¹ DM (Meldor and Timbale cultivars), while the average DMI content of all cultivars was 2.71 g kg⁻¹ DM (average by years). In terms of harvesting stage × cultivar, Fasta and Melissa recorded the highest DMI content. However, when averaged across cultivars, DMI value decreased gradually with advanced stage of development.

Relative feed value

In 2019, when averaged across alfalfa cultivars, plants harvested at 10% flowering stage exhibited significantly higher RFV 5.06 and 20.54 times higher than those at the 50% and 100% flowering stages, respectively (Table 2). While Fasta recorded highest RFV at 10% and 50% flowering stages, it significantly declined at 100% flowering stage.

In 2020, the value for RFV ranged from 85.25% to 195.06% (mean for year: 125.24%). Fasta cultivar harvested at 10% flowering stage ranked first (195.06%) and Timbale harvested at 100% flowering stage ranked as the last (85.25%). Overall, the highest and lowest RFV in 2019 were observed in Melissa harvested at 50% flowering stage (175.33%) and Timbale harvested at 100% flowering stage (95%), respectively. In 2020, at 10% flowering stage all cultivars exhibited the highest RFV compared to the other two harvesting stages.

Metabolism energy

In 2019, the cultivars of Fasta and Melissa exhibited higher ME values than other cultivars (Table 5). In 2020, the Fasta (9.29 MJ) ranked as the first followed by Hamadani and Melissa (8.42 MJ and 8.40 MJ). When averaged across cultivars, the value of the ME in 2019 was slightly higher than in 2020 (some data not shown). At the 10% flowering stage, the highest and lowest ME values were recorded in Fasta (10.29 MJ) and Timbale (6.61 MJ), respectively (Table 6). However, Fasta and Melissa ranked as the first when plants harvested at 50% and 100% flowering stage. Average across the cultivars, the value of ME decreased with the advancement of growth stage from 10% to 100% flowering stage.

Net Energy for Lactation

Averaging all cultivars (Table 2), NEL value reported higher at 100% flowering stage in 2019 than during any other harvested period. In contrast, in 2020 NEL value decreased at 50% flowering stage compared to 10% flowering stage then increased at 100% flowering stage. In 2019, among all the treatments the highest NEL values were observed in Fasta cultivar harvested at 50% (0.76 Mcal kg⁻¹) and 100% (0.76 Mcal kg⁻¹) flowering stage. However, in 2020 the highest NEL recorded in Fasta harvested at 100% flowering stage (0.75 Mcal kg⁻¹). Although the lowest NEL in 2019 was observed in Meldor harvested at 10% flowering stage but lowest NEL in 2020 recorded in Meldor harvested at 50% flowering stage (0.55 Mcal kg⁻¹). Overall, Fasta recorded the highest value of NEL (on average across the harvesting stage) over the two years.

Discussion

Harvesting time of forage quality has a significant effect on yield quality with most quality traits declining with the advancement of growth stage. In this study all cultivars achieved maximum levels of leaf/stem ratio at 10% flowering stage which could be attributed to advancing of plant growth stage likely due to increased photosynthetic activity and reduced stem lignification at earlier growth stages. Likewise, with advancement of mortality

and emergence of flowers, the LAI decreased continuously, while the total dry matter tended to stabilize (some data not shown). Alfalfa stems are less digestible than leaves [13], thus, enhancement in the proportion of leaves to stem ratio led to remarkable improvement in forage quality. Indeed, a positive and significant correlation was achieved between leaf/stem ratio and DMD ($r=0.76^{**}$ and $r=0.81^{**}$, 2019 and 2020, respectively) (Table 7). These results indicated that higher DMD was due to higher leaf fraction rather than proportion of stem which changes with plant maturity.

At each harvesting stage, forage quality displayed significant differences. As the crop progressed from 10% flowering to 100% flowering stage, most of the traits increased. As plants aged, the lignification of cell wall and fiber increased, which reduced the organic matter mineral composition, ash concentration, digestibility and quality of protein [12]. Errickson and Huang [14] stated that growth parameters such as LAI is directly attributed to total dry weight because an increase in LAI (photosynthetic capacity), affects yield performance. Also, this situation may be related to increase in stem more than leaves during advancement in maturity. Given that leaves are the primary contributors to protein in plants [15], foliage is an essential component of forage crops. An increase in rate of stem with advancement in maturity was observed by Liu et al. [16] and Ajoudani et al. [17]. Stems containing higher proportion of water soluble carbohydrates which convert into structural carbohydrates as plants age [18] this conversion is the reason for lower digestibility of alfalfa plants. Despite the lower content of crude protein in mature plants, the amount of protein per unit area decreased with advanced maturity which is associated with higher biomass production per land area [19].

Favorable quality traits (lower fiber content and higher protein content) were different in each harvesting stage and environment (year and seasonal temperature) had significant effect on them. Differences in the protein and fiber value depend on genetic factors, development stage and their interaction [10]. Besides, alfalfa cultivars seem to show different capabilities of adaptability to this region depending on the harvesting stage. Indeed, a modification effect was noticed during the last harvesting stage. In terms fiber and protein, harvesting stage can change the crude protein content of forage [3,20,21]. Crude protein is the most important parameter for forage quality [22]. Based on our results, the first harvest (which occurred in June) is typical of high crude protein due to high portion of leaf in plant canopy. Then the quality decreased in the second and third harvest stage because of accumulation of cellulose and lignin in vascular cells with advancing plant maturity. It has been reported that the concentration of protein decreases as plants mature due to the cross-linking of lignin with cellulose and hemicellulose [23]. It is worth noticing that improving forage quality could impact herbage yield. For instance, forage harvested at 10% flowering had high DMD and low total dry matter (some data not shown). This result may be related to the reduction in main stem length [24]. In addition, many researchers documented that lower DMD and DMI in mature plants related to higher cell wall substances [25]. It seems that the earlier maturity harvest stage (10% flowering) had high protein and DMD but repeated harvest at 50% and 100% flowering (late summer month) will decrease forage quality primarily due to rapid values of lignin and fiber storage [26,27]. Foster et al. [28] stated that forage quality as well as crude protein and DMD decreased with increasing alfalfa production. However, our results revealed that there is cultivars variability in quality traits which means that cultivars selection must be considered with environmental effects as well as harvesting stage. The other reason for lower protein in aged plants could be related to nutrient content which does not match the yield performance, therefore, the concentration of crude protein diluted [29-31]. Besides, the lower level of protein at maturity stage might be the reason of loss of leaves and higher rate of stem in total canopy [32,33]. The concentration of crude protein in plant cells decreased due to the function of lignin accumulation [34,35].

The lowest NDF and ADF were obtained from the first harvest stage or/and cultivars of *Fasta*, it can be related to high correlation with leaf area index and leaf/stem ratio in each year (Table 7). Braun et al. [27] showed that quality aspect of herbage crops is connected to leaf/stem ratio. In this study the highest crude fiber, ADF and NDF were achieved in the last harvested stage, which occurred in the late summer months. Seasonal impacts are due to changes in temperature, solar radiation and photoperiod (day length) [36,37]. In addition, plants harvested at 100% flowering stage had lower leaf water content, lower leaf to stem ratio and higher rate of fiber accumulation. Although, harvesting at 10% flowers (earlier maturity harvests) had higher quality but repeated harvests at 50% and 100% flowering will reduce herbage forage quality. At the beginning of flowering, alfalfa cultivars had the most nutritional status (forage quality) and low yield performance, while they do not have high quality in the maturity state. This could be due to the increment of carbohydrates and reduction in protein density, forage metabolism energy, DMD and RFV which happened by plant maturity [38]. However, producers may avoid the improvement of forage quality in favor of higher dry matter production, therefore delayed harvest may be a suitable option. Liu et al. [16] suggested that the amount of water-soluble carbohydrates changed in structure during the maturity period to make lignin, cellulose and hemicellulose to increase strength and protective tissues. In fact, low carbohydrate levels in the beginning of the flowering are due to low stem to leaf ratio. Therefore, the plant at the 10% flowering stage has more young cells which are thin and have elegant cellular walls, leading to lower ADF and NDF. As the growth period progress, the cellulose walls become thicker and stronger resulting in higher ADF and NDF levels. For high-quality forage, it is desirable for NDF and ADF contents to be as low as possible. Tlahig et al. [13] documented that higher lignin content leads to lower DMD. Additionally, NDF and ADF increased with plant age which is attributed to higher lignin content and lower nutritional value. It can be concluded that the formation of secondary cell walls (thickening) during advancing growth stage leads to synthesis and accumulation of lignin thus, cell wall thickening which occurred in aged plants affected lignin content of forage.

Conclusion

Our results confirm that the optimal time for alfalfa harvest is at the mid-flowering stage, balancing both yield and forage quality. Advancing the cutting time may improve yield production but can reduce forage quantity. Additionally, harvesting in August (100% flowering stage) is not recommended in this region of Iran due to shorter day length and cooler temperatures, which make forage drying difficult. Therefore, it is crucial to avoid excessive delays in harvesting to preserve forage quality. Our findings also indicate that in arid and semi-arid regions, the cultivars *Fasta* and *Meldor* meet all forage quality standards. These results could be valuable for future research, particularly in evaluating the potential of these cultivars

for similar regions or in breeding programs aimed at improving the yield of native cultivars. Furthermore, early harvesting may be an effective strategy to enhance forage quality, as alfalfa harvested at the 10% flowering stage contains higher levels of nutritive constituents compared to harvesting at full bloom (100% flowering stage).

Abbreviations

- PCA
Principal Component Analysis
- L/S
leaf to stem ratio
- LAI
leaf area index
- CP
crude protein
- CF
crude fiber
- NDF
neutral detergent fiber
- ADF
acid detergent fiber
- DMD
dry matter digestibility
- TDN
total digestible nutrients
- DMI
dry matter intake
- RFV
relative feed value
- ME
metabolism energy
- NEL
net energy for lactation
- DM
dry matter
- MJ
Megajoules

Declarations

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Declaration of interest statement: The authors declare that they have no conflict of interest.

Ethics and consent to participate

All plant specimens collected with permissions.

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Author Contribution

Conceptualization and writing—original draft preparation: A.K.; methodology and formal analysis: B.S.; investigation, B.Z.; data curation, H.M.; writing—review and editing, P.M. All authors have read and agreed to the published version of the manuscript.

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Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request."

References

1. Rah Khosravani AT, Mansourifar C, Modarres-Sanavy SAM, Asilan KS, Keshavarz H. Effects of sowing date on physiological characteristics, yield and yield components for different maize (*Zea mays* L.) hybrids. *Notulae Science Biologiae*. 2017;9(1): 143-147. <https://doi.org/10.15835/nsb919913>.
2. Moradi-Ghahderijani M, Jafarian S, Keshavarz H. Alleviation of water stress effects and improved oil yield in sunflower by application of soil and foliar amendments. *Rhizosphere*. 2017; 4: 54-61. <http://dx.doi.org/10.1016/j.rhisph.2017.06.002>.
3. Quinby MP, Nave RLG, Bates GE, McIntosh D. Harvest interval effects on the persistence and productivity of alfalfa grown as a monoculture or in mixtures in the southeastern United States. *Crop, Forage & Turfgrass Management*. 2020;6(1): e20018. <https://doi.org/10.1002/cft2.20018>.
4. Narimani Y, Taleshi K, Khorgami A, Vafaei SH. Cereal-Based Intercropping Systems Improve Yield Advantage and Crop Yield Under Superabsorbent Application in Semiarid Conditions. *Legume Science*. 2024;6: e70002. <https://doi.org/10.1002/leg3.70002>.
5. Xu Z, Heuschele DJ, Lamb JFS, Jung HJG, Samac DA. Improved Forage Quality in Alfalfa (*Medicago sativa* L.) via Selection for Increased Stem Fiber Digestibility. *Agronomy*. 2023; 13(3): 770. <https://doi.org/10.3390/agronomy13030770>.
6. Karami S, Modarres-Sanavy SAM, Ghanehpour S, Keshavarz H. Effect of foliar zinc application on yield, physiological traits and seed vigor of two soybean cultivars under water deficit. *Notulae Scientia Biologicae*. 2016;8 (2): 181-191. <https://doi.org/10.15835/nsb.8.2.9793>.
7. Keshavarz Mirzamohammadi H, Modarres-Sanavy SAM, Sefidkon F, Mokhtassi-Bidgoli A, Mirjalili MH. Irrigation and fertilizer treatments affecting rosmarinic acid accumulation, total phenolic content, antioxidant potential and correlation between them in peppermint (*Mentha piperita* L.). *Irrigation Science*. 2021a;39: 671-683: <https://doi.org/10.1007/s00271-021-00729-z>
8. Mariotti F, Tomé D, Patureau Mirand P. Converting Nitrogen into Protein—Beyond 6.25 and Jones' Factors. *Critical Reviews in Food Science and Nutrition*. 2008;48(2): 177-184, <https://doi.org/10.1080/10408390701279749>
9. Mertens DR. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *J. AOAC Int*. 2002; 85:1217–1240.
10. Batista PSC, Carvalho AJ, Portugal AF, Bastos EA, Cardoso MJ, Torres LG, Mingote Júlio MP, De Menezes CB. Selection of sorghum for drought tolerance in a semi-arid environment. *Genetics and Molecular Research*. 2019; 18(1): gmr18194. <https://doi.org/10.4238/gmr18194>.
11. Lithourgidis AS, Vasilakoglou IB, Dordas CA, Yiakoulaki MD. Forage yield and quality of common vetch mixtures with oat and triticale in two seeding ratios. *Field Crop Research*. 2006; 99: 106-113.
12. Keshavarz Mirzamohammadi H, Tohidi-Moghadam HR, Hosseini SJ. Is there any relationship between agronomic traits, soil properties and essential oil profile of peppermint (*Mentha piperita* L.) treated by fertiliser treatments and irrigation regimes? *Annual Applied Biology*. 2021b; 179 (3): 331-344. <https://doi.org/10.1111/aab.12707>.
13. Tlahig S, Karmous I, Gorai M, Jaouadi T, Loumerem. Effect of Cutting Time on the Performance of Alfalfa (*Medicago sativa* L.) Genotypes Cropped in Arid Environment. *Polish Journal of Environmental Studies*. 2021;30 (2): 1817-1829. <https://doi.org/10.15244/pjoes/124757>.
14. Errickson W, Huang B. Effects of rhizobacteria producing deaminase enzymes for aminocyclopropane-1-carboxylate on drought tolerance and post-stress recovery in creeping bentgrass under field conditions. *Crop, Forage & Turfgrass Management*. 2024; 10: e70013. <https://doi.org/10.1002/cft2.70013>.
15. Keshavarz H, Seify SM, Sabourifar H. Manganese and zinc effect on yield, fatty acid ratio, and maternal seeds germination of canola cultivars upon late-season drought stress. *Journal of Plant Nutrition*. 2024; 47(14): 2216-2231. <https://doi.org/10.1080/01904167.2024.2338746>.
16. Liu X, Li D, Ge Q, Yang B, Li S. Effects of harvest period and mixed ratio on the characteristic and quality of mixed silage of alfalfa and maize. *Animal Feed Science and Technology*. 2023; 306: 115796. <https://doi.org/10.1016/j.anifeedsci.2023.115796>.
17. Ajoudani MR, Sayfzadeh S, Valadabadi SA, Shahsavari N, Zakerin H. Response of forage sorghum cultivars to different water availability. *Crop, Forage & Turfgrass Management*. 2024;10: e70008. <https://doi.org/10.1002/cft2.70008>.
18. Keshavarz H, Sadegh Ghol Moghadam R. Seed priming with cobalamin (vitamin B12) provides significant protection against salinity stress in the common bean. *Rhizosphere*. 2017;3: 143-149. <https://doi.org/10.1016/j.rhisph.2017.04.010>.
19. Keshavarz H, Modarres-Sanavy SAM, Mahdipour Afra M. Organic and chemical fertilizer affected yield and essential oil of two mint species. *Journal of Essential Oil-Bearing Plants*. 2018;21(6): 1674-1681. <https://doi.org/10.1080/0972060X.2018.1497545>.
20. Haghighi-Hasanalideh A, Hojati M. Enhancing yield and nitrogen use efficiency of *Brassica Napus* L. using an integrated fertilizer management. *Advance Environmental Biology*. 2012; 6 (2): 641-647.

21. Akbari GA, Hojati M, Modarres-Sanavy SAM, Ghanati F. Exogenously applied hexaconazole ameliorates salinity stress by inducing an antioxidant defense system in *Brassica napus* L. plants. *Pesticide Biochemistry and Physiology*. 2013;100(3): 244-250. <https://doi.org/10.1016/j.pestbp.2011.04.008>.
22. Keshavarz H. Study of water deficit conditions and beneficial microbes on the oil quality and agronomic traits of canola (*Brassica napus* L.). *Grasas Y Aceites*. 2020;71(3): e373. <https://doi.org/10.3989/gya.0572191>.
23. Yuan N, Sun L, Du S, Ge G, Wang Z, Li Y, Bao J, Zhao M, Si Q, Hao J, Jia Y. Effects of Harvesting Period and Storage Duration on Volatile Organic Compounds and Nutritive Qualities of Alfalfa. *Agriculture*. 2022;12(8):1115. <https://doi.org/10.3390/agriculture12081115>.
24. Xu X, Min D, McDonald I. Effects of harvest intervals and seeding rates on dry matter yield and nutritive value of alfalfa cultivars. *Journal of Animal Science and Technology*. 2021; 63: 1098-1113. <https://doi.org/10.5187/jast.2021.e97>.
25. Monirifar H, Mirmozaffari Roudsari A, Ghassemi S, Tavasolee A. Harvest Time and Cultivar Effects on Growth, Physiological Traits, Yield and Quality of Alfalfa in Saline Condition. *International Journal of Plant Production*. 2020;14: 453–462. <https://doi.org/10.1007/s42106-020-00096-3>.
26. Keshavarz H, Khodabin G. The role of uniconazole in improving physiological and biochemical attributes of bean (*Phaseolus vulgaris* L.) subjected to drought stress. *Journal of Crop Science and Biotechnology*. 2019; 22(2): 161-168. <https://doi.org/10.1007/s12892-019-0050-0>.
27. Braun RC, Straw CM, Soldat DJ, Bekken MA, Patton AJ, Lonsdorf EV, Horgan BP. Strategies for reducing inputs and emissions in turfgrass systems. *Crop, Forage & Turfgrass Management*. 2023; 9(1): e20218. <https://doi.org/10.1002/cft2.20218>.
28. Foster A, Biligetu B, Malhi SS, Gill KS, Mollison B, Leach D. Harvest Time and Fertility Effects on Yield and Quality of Forage from Alfalfa, Hybrid Bromegrass and Their Mixture. *Agricultural Sciences*. 2021; 12: 325-338. <https://doi.org/10.4236/as.2021.124021>.
29. Seify SM, Madani H, Pourdad SS, Nour-Mohammadi G, Changizi M. Impact of deficit irrigation on the physiological and agronomic traits of 24 safflower (*Carthamus tinctorius* L.) genotypes grown in Iran. *Oilseeds and fats, Crops and Lipids*. 2023;30: 27. <https://doi.org/10.1051/ocl/2023027>.
30. Jalilian S, Madani H, Vafaie-Tabar M, Sajedi NA. Plant density influences yield, yield components, lint quality and seed oil content of cotton genotypes. *Oilseeds and fats, Crops and Lipids*. 2023;30: 12. <https://doi.org/10.1051/ocl/2023013>.
31. Hosseini SJ, Tahmasebi-Sarvestani Z, Mokhtassi-Bidgoli A, Keshavarz H, Kazemi Sh, Khalvandi M, Pirdashti H, Bovand F, Abassian A. Do various levels of salinity change chlorophyll fluorescence, nutrient uptake, and physiological characteristics of *Mentha* ecotypes?" *Industrial Crops & Products*. 2023a; 203: 117199. <https://doi.org/10.1016/j.indcrop.2023.117199>.
32. Gholamhoseini M, Ghalavand A, Khodaei-Joghan A, Dolatabadian A, Zakikhani H, Farmanbar, E. Zeolite-amended cattle manure effects on sunflower yield, seed quality, water use efficiency and nutrient leaching. *Soil and Tillage Research*. 2013; 126: 193-202. <http://dx.doi.org/10.1016/j.still.2012.08.002>.
33. Keshavarz H, Modarres-Sanavy SAM, Sadegh Ghol Moghadam R. Impact of foliar application with salicylic acid on biochemical characters of canola plants under cold stress condition. *Notulae Science Biologiae*. 2016; 8(1): 98-105. <https://doi.org/10.15835/nsb.8.1.9766>.
34. Gholamhoseini M, AghaAlikhani M, Dolatabadian A, Khodaei-Joghan A, Zakikhani H. Decreasing nitrogen leaching and increasing canola forage yield in a sandy soil by application of natural zeolite. *Agronomy Journal*. 2012; 104: 1467-1475. <https://doi.org/10.2134/agronj2012.0145>.
35. Hosseini SJ, Tahmasebi-Sarvestani Z, Mokhtassi-Bidgoli A, Keshavarz H, Kazemi Sh, Khalvandi M, Pirdashti H, Hashemi-Petroudi SH, Nicola S. Functional Quality, Antioxidant Capacity and Essential Oil Percentage in Different Mint Species Affected by Salinity Stress. *Chemistry and Biodiversity*. 2023b; 20: e202200247. <https://doi.org/10.1002/cbdv.202200247>.
36. Sabourifard H, Estakhr A, Bagheri M, Hosseini SJ, Keshavarz H. The quality and quantity response of maize (*Zea mays* L.) yield to planting date and fertilizers management. *Food Chemistry Advances*. 2023;2: 100196. <https://doi.org/10.1016/j.focha.2022.100196>.
37. Yousefzadeh S, Keshavarz H, Modares-Sanavy SAM. Effects of ecological agriculture approaches on dragonhead (*Dracocephalum moldavica* L.) productivity and oil yield. *Arabian Journal of Geoscience*. 2023;16: 373. <https://doi.org/10.1007/s12517-023-11461-7>.
38. Keshavarz H, Hosseini SJ, Sedibe MM, Achilonu MC. Arbuscular mycorrhizal fungi used to support Iranian barley (*Hordeum vulgare* L.) cultivated on cadmium contaminated soils. *Applied Ecology and Environmental Research*. 2021;20(1): 43-53. http://dx.doi.org/10.15666/aeer/2001_043053.

Tables

Table 1- Combined analysis of variance on leaf/stem ratio, leaf area index and some forage quality traits of alfalfa (*Medicago sativa* L.) as affected by harvesting stage and cultivar treatments.

Source of variation	df	L/S	LAI	CP	CF	NDF	ADF	DMD	TDN	DMI	RFV	ME	NEL
Year	1	0.08*	4.49**	0.18ns	18.1*	8.9ns	181.4*	5.78ns	302*	0.35ns	953.1ns	0.1ns	0.2*
Year × Replication	4	0.004	0.21	0.18	1.05	23.5	17.2	4.81	28.6	0.11	151.6	0.1	0.002
Stage	2	0.2**	10.03**	0.79**	47.5**	577.7**	227.3**	167.6**	378.3**	2.25**	8690**	4.8**	0.03**
Cultivar	5	0.075**	0.75**	2.19**	16.9**	364.9**	333.4**	407.3**	554.8**	1.5**	9137**	11.7**	0.04**
Year × Stage	2	0.04**	0.94**	0.01ns	10.1**	51.5**	51.3**	7.5ns	85.4**	0.22**	525**	0.2ns	0.007**
Year × Cultivar	5	0.009*	0.07**	0.13ns	2.08*	12.5**	6.57ns	10.9*	10.9ns	0.07**	421.7**	0.3*	0.0009 ^{ns}
Stage × Cultivar	10	0.07**	0.94**	0.09ns	17.7**	5.55**	12.7**	107.2**	21.2**	0.02**	388.9**	3.09**	0.001**
Year × Stage × Cultivar	10	0.02**	0.12**	0.02ns	4.6**	2.65ns	9.79*	3.62ns	16.2*	0.01ns	127.1*	0.1ns	0.001*
Error	68	0.003	0.01	0.1	0.84	2.08	3.8	3.6	6.3	0.009	54.3	0.1	0.0005
CV (%)		4.6	4.7	1.9	4.6	3.2	6	3.2	4.2	3.5	5.7	4	3.5

L/S: leaf to stem ratio; LAI: leaf area index; CP: crude protein; CF: crude fiber; NDF: neutral detergent fiber; ADF: acid detergent fiber; DMD: dry matter digestibility; TDN: total digestible nutrients; DMI: dry matter intake; RFV: relative feed value; ME: metabolism energy; NEL: net energy for lactation. ns, ** and * indicate no significance and significance at *P* levels of 0.01 and 0.05, respectively.

Table 2- Three way interaction of year, harvesting stage and cultivar on studied traits of alfalfa (*Medicago sativa* L.).

Year	Harvesting Stage	Cultivar	L/S	LAI	CF (%)	ADF (g kg ⁻¹ DM)	TDN (g kg ⁻¹ DM)	RFV (%)	NEL (Mcal kg ⁻¹ DM)
2019	10% flowering	Fasta	1.54 a	3.76 a	17.40 def	23.33 i	71.25 a	171.72 a	0.67 de
		Timbale	1.54 a	3.51 b	17.84 cdef	27.66 gh	65.66 bc	141.99 c	0.62 fg
		Melissa	1.54 a	3.12 c	19.65 b	31.33 ef	60.93 de	124.29 efg	0.56 i
		Hamadani	1.26 bcd	2.75 d	19.35 bc	25.00 hi	69.10 ab	170.33 a	0.71 bc
		Meldor	1.32 b	2.50 e	16.90 f	29.33 fg	63.51 cd	139.80 cd	0.63 efg
		Sovrana	1.24 bcde	2.29 ef	17.05 ef	34.66 cde	56.63 efg	112.33 h	0.58 hi
	50% flowering	Fasta	1.23 bcdef	2.13 fg	18.45 bcdef	23.66 i	70.82 a	154.79 b	0.76 a
		Timbale	1.32 b	2.24 f	19.8 b	28.00 fgh	65.23 bcd	128.15 def	0.70 cd
		Melissa	1.28 bc	2.28 ef	19.3 bc	33.00 de	58.78 ef	105.99 hi	0.65 ef
		Hamadani	1.14 ef	1.97 gh	17.10 ef	28.66 fg	64.37 cd	175.33 a	0.70 cd
		Meldor	1.28 bc	2.12 fg	19.25 bc	36.66 bc	54.05 gh	140.35 cd	0.60 gh
		Sovrana	1.17 def	1.95 gh	17.60 def	35.66 bcd	55.34 fgh	112.27 gh	0.61 fgh
	100% flowering	Fasta	1.16 def	2.11 fg	23.10 a	31.33 ef	60.93 de	135.12 cde	0.76 a
		Timbale	1.19 cdef	2.07 fg	23.10 a	35.00 cd	56.20 fg	112.04 gh	0.71 bc
		Melissa	1.31 b	2.26 f	21.80 a	40.66 a	48.89 i	94.14 ij	0.67 de
		Hamadani	1.29 bc	2.14 fg	18.9 bcd	27.66 gh	65.66 bc	134.63 cde	0.74 ab
		Meldor	1.12 e	1.78 h	19.9 b	34.00 cde	57.49 efg	116.54 gh	0.69 cd
		Sovrana	1.13 ef	1.96 gh	18.6 bcde	38.66 ab	51.47 hi	91.26 j	0.63 efg
2020	10% flowering	Fasta	1.76 a	4.24 a	17.30 i	24.00 g	70.39 a	195.06 a	0.68 bc
		Timbale	1.53 b	4.07 b	17.80 hi	29.33 f	63.51 b	161.63 b	0.63 de
		Melissa	1.21 ghi	3.35 c	19.45 defg	35.66 c	55.34 e	127.42 efg	0.58 fg
		Hamadani	1.24 fghi	2.93 e	20.00 def	27.66 f	65.66 b	149.11 c	0.65 cd
		Meldor	1.27 efgh	2.96 e	20.45 cde	32.66 de	59.21 cd	126.16 efg	0.65 cd
		Sovrana	1.22 ghi	2.42 g	18.45 ghi	37.33 bc	53.19 ef	100.92 jk	0.56 g
	50% flowering	Fasta	1.37 cd	2.41 g	20.55 cd	29.00 f	63.94 b	145.24 c	0.69 b
		Timbale	1.44 bc	2.68 f	21.70 bc	36.00 bc	54.91 ef	118.62 hi	0.61 ef
		Melissa	1.48 b	2.74 f	2.25 bc	41.33 a	48.03 g	101.36 jk	0.55 g
		Hamadani	1.29 defg	3.03 de	19.35 defg	32.33 de	59.64 cd	143.85 cd	0.65 cd
		Meldor	1.32 def	3.18 cd	19.90 def	36.66 bc	54.05 ef	120.56 fgh	0.60 ef
		Sovrana	1.25 fgh	3.22 c	18.85 fgh	40.33 a	49.32 g	99.83 jk	0.56 g
	100% flowering	Fasta	1.15 i	2.31 gh	26.40 a	30.00 ef	62.65 bc	137.47 cde	0.75 a
		Timbale	1.18 hi	2.22 hi	23.05 b	34.66 cd	56.63 de	110.87 hij	0.69 b
		Melissa	1.29 defg	2.28 gh	18.15 ghi	38.66 ab	51.47 fg	91.39 kl	0.61 ef
		Hamadani	1.33 def	2.06 ij	18.65 fghi	32.33 de	59.64 cd	132.05 def	0.71 b
		Meldor	1.36 cde	2.05 j	19.15 efgh	32.66 de	59.21 cd	107.53 ij	0.65 cd
		Sovrana	1.23 ghi	2.15 hij	18.40 ghi	40.33 a	49.32 g	85.25 l	0.59 ef

DM: dry matter; L/S: leaf to stem ratio; LAI: leaf area index; CF: crude fiber; ADF: acid detergent fiber; TDN: total digestible nutrients; RFV: relative feed value; NEL: net energy for lactation. Means within each column of each section and each row of year effect followed by the same letter are

not significantly different by LSD test at $P \leq 0.05$.

Table 3- Mean comparison of main effect of harvesting stage and cultivar treatments on crude protein of alfalfa (*Medicago sativa* L.).

Harvesting stage	Crude protein (%)
10% flowering	1.38 a
50% flowering	1.30 b
100% flowering	1.23 c
Cultivar	
Fasta	16.85 c
Timbale	17.52 a
Melissa	17.22 b
Hamadani	17.31 ab
Meldor	16.55 d
Sovrana	17.18 b

Means followed by the same letter are not significantly different by LSD test at $P \leq 0.05$. Section are separated by blank row.

Table 4- Two way interaction of year and harvesting stage treatments on studied traits of alfalfa (*Medicago sativa* L.).

Year	Harvesting stage	Neutral detergent fiber (g kg ⁻¹ DM)	Dry matter intake (g kg ⁻¹ DM)
2019	10% flowering	40.94 b	2.97 a
	50% flowering	40.77 b	2.97 a
	100% flowering	47.38 a	2.55 b
2020	10% flowering	40.27 c	3.01 a
	50% flowering	44.88 b	2.69 b
	100% flowering	49.38 a	2.45 c

DM: dry matter; Means within each column of each section and each row of year effect followed by the same letter are not significantly different by LSD test at $P \leq 0.05$.

Table 5- Two way interaction of year and cultivar treatments on studied traits of alfalfa (*Medicago sativa* L.).

Year	Cultivar	NDF (g kg ⁻¹ DM)	DMD (g kg ⁻¹ DM)	DMI (g kg ⁻¹ DM)	ME (MJ)
2019	Fasta	39.22 e	64.55 a	3.07 b	8.97 a
	Timbale	44.55 c	60.77 b	2.70 d	8.33 b
	Melissa	48.88 a	56.55 c	2.46 e	7.61 c
	Hamadani	37.0 f	62.77 a	3.28 a	8.67 a
	Meldor	41.44 d	58.44 c	2.92 c	7.93 c
	Sovrana	47.11 c	52.88 d	2.56 e	6.99 d
2020	Fasta	39.33 c	66.44 a	3.07 a	9.29 a
	Timbale	44.44 b	61.33 b	2.72 b	8.42 b
	Melissa	49.88 a	56.66 c	2.41 c	7.63 c
	Hamadani	40.44 c	61.22 b	2.99 a	8.40 b
	Meldor	44.88 b	56.55 c	2.70 b	7.61 c
	Sovrana	50.11 a	51.0 d	2.41 c	6.67 d

DM: dry matter; MJ: Megajoules; NDF: neutral detergent fiber; DMD: dry matter digestibility; DMI: dry matter intake; ME: metabolism energy. Means within each column of each section and each row of year effect followed by the same letter are not significantly different by LSD test at $P \leq 0.05$.

Table 6- Two way interaction of harvesting stage and cultivar treatments on studied traits of alfalfa (*Medicago sativa* L.).

Harvesting stage	Cultivar	NDF (g kg ⁻¹ DM)	DMD (g kg ⁻¹ DM)	DMI (g kg ⁻¹ DM)	ME (MJ)
10% flowering	Fasta	36.83 de	72.33 a	3.26 ab	10.29 a
	Timbale	41.83 c	68.00 b	2.88 c	9.56 b
	Melissa	46.83 a	63.33 c	2.56 d	8.76 c
	Hamadani	35.00 e	59.66 d	3.44 a	8.14 d
	Meldor	38.83 d	55.33 e	3.09 b	7.40 e
	Sovrana	44.33 b	50.66 f	2.71 cd	6.61 f
50% flowering	Fasta	38.66 c	62.16 a	3.11 a	8.56 a
	Timbale	43.50 b	57.50 b	2.76 b	7.77 b
	Melissa	47.33 a	52.66 c	2.54 c	6.95 c
	Hamadani	37.50 c	63.83 a	3.22 a	8.85 a
	Meldor	42.50 b	59.33 b	2.83 b	8.08 a
	Sovrana	47.50 a	54.00 c	2.53 c	7.18 c
100% flowering	Fasta	42.33 c	62.00 a	2.83 a	8.54 a
	Timbale	48.16 b	57.66 b	2.49 b	7.80 b
	Melissa	54.00 a	53.83 c	2.22 c	7.15 c
	Hamadani	43.66 c	62.50 a	2.75 a	8.62 a
	Meldor	48.16 b	58.83 b	2.49 b	7.83 b
	Sovrana	54.00 a	51.16 d	2.22 c	6.69 d

DM: dry matter; NDF: neutral detergent fiber; DMD: dry matter digestibility; DMI: dry matter intake; ME: metabolism energy. Means within each column of each section and each row of harvesting stage effect followed by the same letter are not significantly different by LSD test at $P \leq 0.05$.

Table 7- Pearson correlation coefficients between studied traits of alfalfa (*Medicago sativa* L.).

		1	2	3	4	5	6	7	8	9	10	11	12
10% flowering	Leaf/stem ratio (1)	1	0.83 **	-0.10 ns	-0.72 **	-0.52 *	-0.71 **	0.81 **	0.71 **	0.52 *	0.82 **	0.81 **	0.71 **
	Leaf area index (2)	0.81 **	1	0.11 ns	-0.57 *	-0.38 ns	-0.69 *	0.94 **	0.69 **	0.38 ns	0.82 **	0.94 **	0.69 **
	Crude protein (3)	0.001 ns	0.20 ns	1	-0.22 ns	0.24 ns	0.11 ns	0.08 ns	-0.11 ns	-0.24 ns	-0.06 ns	0.08 ns	-0.11 ns
	Crude fiber (4)	0.06 ns	0.15 ns	0.046 ns	1	0.03 ns	0.32 ns	-0.54 *	-0.32 ns	-0.02 ns	-0.4 ns	-0.54 *	-0.32 ns
	Neutral detergent fiber (5)	0.35 ns	0.14 ns	0.25 ns	0.10 ns	1	0.74 **	-0.44 ns	-0.74 **	-0.99 **	-0.78 **	-0.44 ns	-0.74 **
	Acid detergent fiber (6)	-0.43 ns	-0.65 **	0.033 ns	-0.15 ns	0.53 *	1	-0.68 **	-0.99 **	-0.74 **	-0.83 **	-0.68 **	-0.99 **
	Dry matter digestibility (7)	0.76 **	0.90 **	0.086 ns	0.21 ns	-0.017 ns	-0.65 **	1	0.68 **	0.45 ns	0.89 **	0.99 **	0.68 **
	Total digestible nutrients (8)	0.43 ns	0.65 **	-0.033 ns	0.15 ns	-0.53 *	0.99 **	0.65 **	1	0.74 **	0.83 **	0.68 **	0.99 **
	Dry matter intake (9)	-0.35 ns	-0.15 ns	-0.24 ns	-0.0006 ns	-0.99 **	-0.51 *	0.025 ns	0.51 *	1	0.80 **	0.45 ns	0.74 **
	Relative feed value (10)	0.18 ns	0.42 ns	-0.14 ns	0.12 ns	-0.78 **	-0.8 **	0.62 **	0.8 **	0.79 **	1	0.89 **	0.83 **
	Metabolism energy (11)	0.76 **	0.90 **	0.08 ns	0.21 ns	-0.017 ns	-0.65 **	0.99 **	0.65 **	0.025 ns	0.62 **	1	0.68 **
	Net energy for lactation (12)	0.43 ns	0.65 **	-0.03 ns	0.15 ns	-0.53 *	0.99 **	0.65 **	0.99 **	0.51 *	0.80 **	0.65 **	1
50% flowering	Leaf/stem ratio (1)	1	-0.55 *	0.12 ns	0.99 **	0.06 ns	0.26 ns	-0.24 ns	-0.26 ns	-0.07 ns	-0.16 ns	-0.24 ns	-0.26 ns
	Leaf area index (2)	0.73 **	1	-0.09 ns	-0.55 *	0.32 ns	0.48 *	-0.32 ns	-0.48 *	-0.32 ns	-0.34 ns	-0.32 ns	-0.48 *
	Crude protein (3)	-0.14 ns	-0.01 ns	1	0.12 ns	0.13 ns	0.07 ns	-0.25 ns	-0.07 ns	-0.13 ns	-0.19 ns	-0.25 ns	-0.07 ns
	Crude fiber (4)	0.99 **	0.73 **	-0.14 ns	1	0.06 ns	0.26 ns	-0.24 ns	-0.26 ns	-0.07 ns	-0.16 ns	-0.24 ns	-0.26 ns
	Neutral detergent fiber (5)	0.42 ns	0.30 ns	0.13 ns	0.42 ns	1	0.78 **	-0.85 **	-0.78 **	-0.99 **	-0.97 **	-0.85 **	-0.78 **
	Acid detergent fiber (6)	0.12 ns	-0.01 ns	-0.36 ns	0.12 ns	0.44 ns	1	-0.84 **	-0.99 **	-0.79 **	-0.85 **	-0.84 **	-0.99 **
	Dry matter digestibility (7)	-0.38 ns	-0.47 *	-0.19 ns	-0.38 ns	-0.85 **	-0.42 ns	1	0.84 **	0.85 **	0.94 **	0.99 **	0.84 **
	Total digestible nutrients (8)	-0.12 ns	0.014 ns	0.36 ns	-0.12 ns	-0.44 ns	-0.99 **	0.42 ns	1	0.79 **	0.85 **	0.84 **	0.99 **
	Dry matter intake (9)	-0.46 ns	-0.3 ns	-0.09 ns	-0.46 ns	-0.99 **	-0.43 ns	0.85 **	0.43 ns	1	0.97 **	0.85 **	0.79 **
	Relative feed value (10)	-0.46 ns	-0.39 ns	-0.13 ns	-0.46 ns	-0.96 **	-0.45 ns	0.95 **	0.45 ns	0.97 **	1	0.94 **	0.85 **
	Metabolism energy (11)	-0.38 ns	-0.47 *	-0.19 ns	-0.38 ns	-0.85 **	-0.42 ns	0.99 **	0.42 ns	0.85 **	0.95 **	1	0.84 **
	Net energy for lactation (12)	-0.12 ns	0.01 ns	0.36 ns	-0.12 ns	-0.44 ns	-0.99 **	0.42 ns	0.99 **	0.43 ns	0.45 ns	0.42 ns	1

100% flowering	Leaf/stem ratio (1)	1	-0.64 **	-0.02 ns	-0.72 **	0.14 ns	0.12 ns	-0.13 ns	-0.12 ns	-0.16 ns	-0.16 ns	-0.13 ns	-0.12 ns
	Leaf area index (2)	0.57 *	1	-0.10 ns	0.54 *	-0.08 ns	-0.04 ns	0.02 ns	0.04 ns	0.1 ns	0.08 ns	0.02 ns	0.04 ns
	Crude protein (3)	0.61 **	0.54 ns	1	-0.28 ns	0.19 ns	0.1 ns	-0.03 ns	-0.1 ns	-0.2 ns	-0.13 ns	-0.03 ns	-0.1 ns
	Crude fiber (4)	0.061 ns	0.25 ns	0.017 ns	1	-0.64 **	-0.54 *	0.54 *	0.54 *	0.65 **	0.62 **	0.54 *	0.54 *
	Neutral detergent fiber (5)	0.06 ns	0.01 ns	0.19 ns	-0.11 ns	1	0.77 **	-0.89 **	-0.77 **	-0.99 **	-0.97 **	-0.89 **	-0.77 **
	Acid detergent fiber (6)	0.001 ns	0.07 ns	0.009 ns	0.01 ns	0.85 **	1	-0.85 **	-0.99 **	-0.76 **	-0.81 **	-0.85 **	-0.99 **
	Dry matter digestibility (7)	0.066 ns	0.15 ns	-0.002 ns	0.17 ns	-0.86 **	-0.82 **	1	0.85 **	0.88 **	0.96 **	0.99 **	0.85 **
	Total digestible nutrients (8)	-0.001 ns	-0.07 ns	-0.009 ns	-0.017 ns	-0.85 **	0.98 **	0.82 **	1	0.76 **	0.81 **	0.85 **	0.99 **
	Dry matter intake (9)	-0.04 ns	0.02 ns	-0.16 ns	0.11 ns	-0.99 **	-0.85 **	0.86 **	0.85 **	1	0.97 **	0.88 **	0.76 **
	Relative feed value (10)	0.007 ns	0.09 ns	-0.09 ns	0.14 ns	-0.97 **	-0.86 **	0.95 **	0.86 **	0.97 **	1	0.96 **	0.81 **
	Metabolism energy (11)	0.06 ns	0.15 ns	-0.002 ns	0.17 ns	-0.86 **	-0.82 **	0.97 **	0.82 **	0.86 **	0.95 **	1	0.85 **
	Net energy for lactation (12)	-0.001 ns	-0.07 ns	-0.009 ns	-0.01 ns	-0.85 **	-0.98 **	0.82 **	0.98 **	0.85 **	0.86 **	0.82 **	1

Means followed by the same letter are not significantly different ($P \leq 0.05$). The correlation coefficients in the upper and down part of the table diameter are belonged to first (2019) and second year (2020), respectively.

Figures

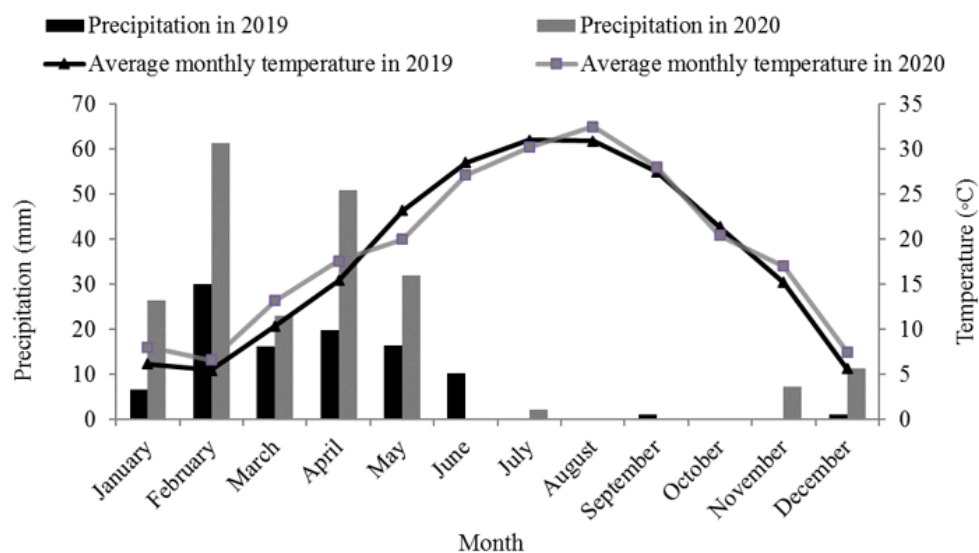


Figure 1

Average temperature and monthly rainfall during the growing seasons in 2019 and 2020

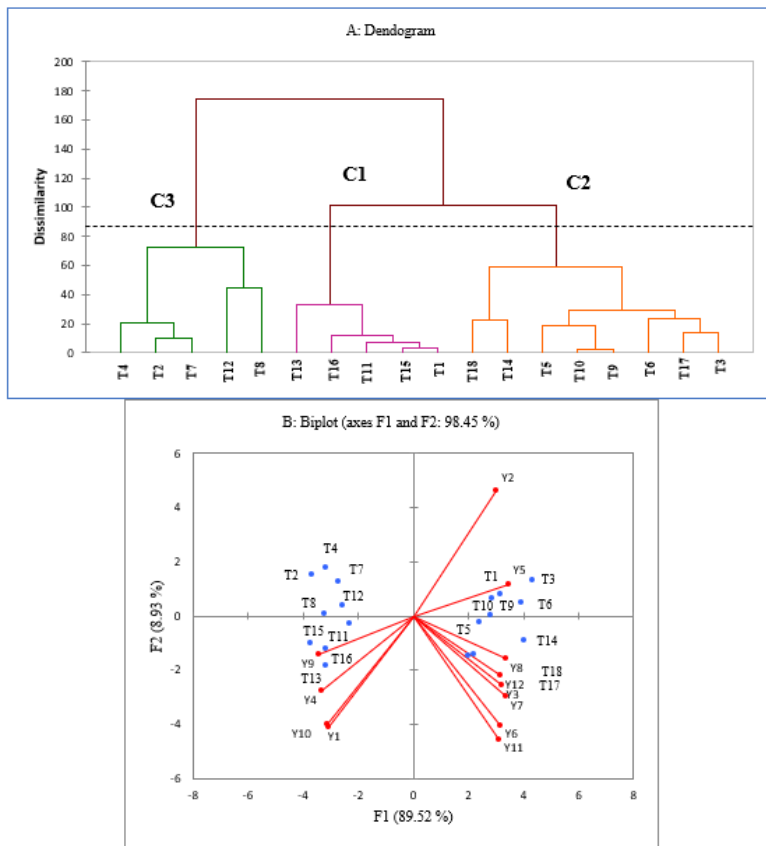


Figure 2

The results of dendrogram based on cluster analysis (A) and Biplot derived from first and second component based on all studied traits (B). leaf to stem ratio (Y1), leaf area index (Y2), crude protein (Y3), crude fiber (Y4), neutral detergent fiber (Y5), acid detergent fiber (Y6), dry matter digestibility (Y7), total digestible nutrients (Y8), dry matter intake (Y9), relative feed value (Y10), metabolism energy (Y11) and net energy for lactation (Y12); T1: 10% flowering+ Fasta, T2: 10% flowering+ Timbale, T3: 10% flowering+ Melissa, T4: 10% flowering+ Hamadani, T5: 10% flowering+ Meldor, T6: 10% flowering+ Sovrana, T7: 50% flowering+ Fasta, T8: 50% flowering+ Timbale, T9: 50% flowering+ Melissa, T10: 50% flowering+ Hamadani, T11: 50% flowering+ Meldor, T12: 50% flowering+ Sovrana, T13: 100% flowering+ Fasta, T14: 100% flowering+ Timbale, T15: 100% flowering+ Melissa, T16: 100% flowering+ Hamadani, T17: 100% flowering+ Meldor, T18: 100% flowering+ Sovrana.