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# Effect of Environment × Cultivar Interaction on Protein and Mineral Contents of Alfalfa (*Medicago sativa* L.) in Central Anatolia, Turkey

(Kesan Interaksi Alam Sekitar × Kultivar ke atas Kandungan Protein dan Mineral Alfalfa (*Medicago sativa* L.) di Anatolia Tengah, Turki)

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ABSTRACT

*Nutritive value and persistence of different alfalfa cultivars, but very little information is available in the literature on mineral concentrations, especially on microelements. This study was carried out to determine crude protein and mineral contents in alfalfa cultivars from two different locations, Ankara and Konya provinces, in Central Anatolia during 2005 and 2007. The experiment was conducted using a randomized complete block design with three replications. Significant differences in many investigated traits were found between cultivars, years, locations and location x year, location x cultivar, year x cultivar, and location x year x cultivar interactions. A crude protein ratio and micro and macro element contents (K, P, Mg, Ca, Mn, Fe, Zn, B, Mo, Cu, Cr, Se and Al) were analyzed in alfalfa on a dry weight basis. The results showed that the protein content ranged 17.23 to 20.71%. The macro element contents ranged 2.06 to 3.95 g kg$^{-1}$ for K, 13.65 to 23.25 g kg$^{-1}$ for P, 1.31 to 2.53 g kg$^{-1}$ for Mg, 6.71 to 22.81 g kg$^{-1}$ for Ca, and 44.3 to 92.7 mg kg$^{-1}$ for Fe, whereas the microelement contents ranged 15.4 to 54.3 mg kg$^{-1}$ for Mn, 24.6 to 121.9 mg kg$^{-1}$ for Zn, 46.4 to 85.4 mg kg$^{-1}$ for B, 0.50 to 6.13 mg kg$^{-1}$ for Mo, 3.13 to 4.17 mg kg$^{-1}$ for Cu, 0.93 to 2.40 mg kg$^{-1}$ for Cr, 0.77 to 1.03 mg kg$^{-1}$ for Se and 31.2 to 57.8 mg kg$^{-1}$ for Al. Significant differences at the $p<0.01$ or $p<0.05$ level in many investigated traits were found between cultivars, years, locations and location × year, location × cultivar, year × cultivar, and location × year × cultivar interactions. In conclusion, due to the fact that the location × year × cultivar interaction was found statistically significant, we suggest that appropriate cultivars are selected for each region.*

*Keywords: Alfalfa; location; macro- and microelement; quality*

ABSTRAK

*Nutrien dan ketahanan kultivar alfalfa mempunyai nilai berbeza tetapi maklumat yang terdapat dalam kajian perpustakaan mengenai kepekatan mineral terutama pada unsur mikro adalah sangat sedikit. Kajian ini dijalankan untuk menentukan kandungan protein mentah dan mineral dalam kultivar alfalfa dari dua lokasi berbeza iaitu di wilayah Ankara dan Konya, di Anatolia Tengah pada tahun 2005 dan 2007. Uji kaji ini dilakukan dengan menggunakan reka bentuk blok lengkap secara rawak dengan tiga ulangan. Perbezaan penting dalam banyak sifat yang dikaji didapati antara kultivar, tahun, lokasi dan lokasi x tahun, lokasi x kultivar, tahun x kultivar dan lokasi x tahun x interaksi kultivar. Nisbah protein kasar dan kandungan unsur mikro dan makro (K, P, Mg, Ca, Mn, Fe, Zn, B, Mo, Cu, Cr, Se dan Al) dianalisis dalam alfalfa berdasarkan berat kering. Keputusan menunjukkan bahawa kandungan protein berkisar antara 17.23 hingga 20.71%. Kandungan unsur makro berkisar antara 2.06 hingga 3.95 g kg$^{-1}$ untuk K, 13.65 hingga 23.25 g kg$^{-1}$ untuk P, 1.31 hingga 2.53 g kg$^{-1}$ untuk Mg, 6.71 hingga 22.81 g kg$^{-1}$ untuk Ca, dan 44.3 hingga 92.7 mg kg$^{-1}$ untuk Fe, manakala kandungan elemen mikro berkisar 15.4 hingga 54.3 mg kg$^{-1}$ untuk Mn, 24.6 hingga 121.9 mg kg$^{-1}$ untuk Zn, 46.4 hingga 85.4 mg kg$^{-1}$ untuk B, 0.50 hingga 6.13 mg kg$^{-1}$ untuk Mo, 3.13 hingga 4.17 mg kg$^{-1}$ untuk Cu, 0.93 hingga 2.40 mg kg$^{-1}$ untuk Cr, 0.77 hingga 1.03 mg kg$^{-1}$ untuk Se dan 31.2 hingga 57.8 mg kg$^{-1}$ untuk Al. Perbezaan yang signifikan di tahap $p <0.01$ atau $p <0.05$ dalam banyak sifat yang dikaji didapati antara kultivar, tahun, lokasi dan lokasi × tahun, lokasi kultivar, tahun kultivar serta lokasi × tahun × interaksi kultivar. Kesimpulannya, lokasi × tahun × interaksi kultivasi didapati secara signifikan, kami mencadangkan bahawa kultivar yang sesuai dipilih untuk setiap rantau.*

*Kata kunci: Alfalfa; kualiti; lokasi ; unsur makro dan mikro*

## INTRODUCTION

At present, both global population and consumption per capita of animal products are rapidly increasing. Consequently, the demand for animal products is also increasing. The meadow and pasture fields available cannot satisfy the growing need for animal feeding (Ozkose 2013) without increasing the harvest area, yield and quality of forage crops.

Alfalfa is a valuable forage crop both worldwide and in Turkey, grown for hay and silage. Its importance is due to a high nutritious value, perennial longevity, multiple harvests per year, a deep root system, high adaptability to warm and cold climates and the ability to increase soil fertility due to nitrogen fixation (Acar et al. 2011; Avcioglu et al. 2009; Ceylan et al. 2009). Not surprisingly, alfalfa is called a queen of forage crops.

The remarkable adaptability of alfalfa allows it to growing cold region such as Siberia and Alaska in the Northern Hemisphere and in a hot climate of North Africa and the Arabian Peninsula (Avci et al. 2013). It is grown in all regions of Turkey (Tongel & Ayan 2010). The different regions have different climates, thus alfalfa cultivars grown in each region vary. Therefore, selection of appropriate cultivars is essential.

All plants depend upon soil for their supply of mineral nutrients and grazing ruminant animals obtain the majority of their mineral nutrients from plants grown on these soils (Khan et al. 2006). Minerals make up a small portion of an animal diet; however, they play an important role in animals' health, growth, and reproduction (Lemus 2013). With regard to quality and quantity of plant and animal production, macro and micro minerals need to be optimal levels (Aslan 2017). Forage mineral concentrations are much more variable than those of protein and energy sources (Lemus 2013). Although the crop yield is still the primary economic factor determining the forage crop value per unit of land area, the forage quality has become a close second (Orloff & Putnam 2007). The quality of alfalfa hay is closely related to its protein and mineral contents (Tongel & Ayan 2010). The alfalfa hay quality and concentrations of mineral elements in alfalfa can be influenced by factors such as harvesting at specific physiological stages, climatic factors, soil conditions, leaf losses during hay production, storage and feeding practices, diseases and insects, weeds, an alfalfa cultivar, the moisture content during storage, a water supply and fertilizer application (Kahraman 2017; Khan et al. 2006; Orloff & Putnam 2007; Scholtz et al. 2009).

Numerous studies have been conducted in different regions of the world on a dry matter yield, nutritive value and persistence of different alfalfa cultivars (Orloff & Putnam 2007), but very little information is available in the literature on mineral concentrations, especially on microelements, in alfalfa cultivars from Central Anatolia, which has a continental climate. Therefore, the aim of this study was to determine protein, macro and microelement contents in the herbage of some alfalfa cultivars grown in Central Anatolia for three years from 2005 to 2007.

## Materials and Methods

### EXPERIMENTAL SITE, SOIL AND WEATHER CONDITIONS

This research was conducted in two locations, namely, Konya and Ankara provinces, Central Anatolia Region, Turkey, during 2005-2007.

The research fields in Konya and Ankara provinces are located at 38°02'01.4"N, 32°30'56.4" E and 39°50'15.1"N, 32°25'13.9" E and at altitudes of 1,130 and 760 m above mean sea level, respectively. Soil samples were taken before sowing and analyzed for certain chemical and physical parameters (Table 1). The soil at the experimental area in Konya has a clay loam texture and is slightly alkaline and low in organic matter, while the soil from the Ankara site has a sandy clay texture and is slightly alkaline (Ulgen & Yurtsever 1974) and low in organic matter according to the Walkley-Black procedure (Kacar 1994).

TABLE 1. Soil characteristics of the experimental area in Konya and Ankara

| Parameters | Values | |
|---|---|---|
| | Konya | Ankara |
| Clay (%) | 27.6 | 35.8 |
| Silt | 31.4 | 18.6 |
| Sand | 41.0 | 45.6 |
| Textural class | Clay loam | Sandy clay |
| pH | 7.8 | 7.6 |
| Organic matter (%) | 1.3 | 1.5 |
| EC (µS $cm^{-1}$) | 181 | 285 |
| $P_2O_5$ (ppm) | 10.74 | 28.66 |
| $K_2O$ (ppm) | 227.4 | 286.92 |
| Zn (ppm) | 6.05 | 1.526 |
| Fe (ppm) | 2.76 | 6.378 |
| Cu (ppm) | 0.87 | 1.59 |
| Mn (ppm) | 3.71 | 15.83 |
| Ca (ppm) | 6134 | 2765 |

The climate of the Konya and Ankara provinces can be defined as semiarid continental. The monthly averages of meteorological data during the experimental seasons (October 2005 and September 2007) are provided in Table 2. According to the meteorological data, the long-term (1975-2005) average rainfall is 323.6 and 389. 1 mm, the average annual temperature is 11.4 and 11.7°C and the average annual relative humidity is 58.0 and 60.5% in the Konya and Ankara provinces, respectively.

### FIELD EXPERIMENT AND PLANT MATERIAL

The experiment was conducted using a randomized complete block design with three replications. The sowings were made in rows of 5 m long, with 0.2 m inter-row space and eight rows per plot. The plot size was 5 × 1.6 m (8 $m^2$). Alfalfa was sown for the field trial at the end of September in Konya and at the end of July in Ankara in 2005. The plots were watered after sowing in order to support seedling emergence. In the study, six different alfalfa (*M. sativa* L.) cultivars (CV) were used as plant material, namely, Kayseri (CV1), Sunter (CV2), Verko (CV3), Hemedan (CV4), Elci (CV5) and Planet (CV6).

Fertilizers were applied to supply 27 kg $ha^{-1}$ of nitrogen (N) and 69 kg $ha^{-1}$ of phosphorus ($P_2O_5$) before sowing and

TABLE 2. Monthly total rainfall, average temperature and average relative air humidity recorded at Konya and Ankara province during the October 2005 and September 2007 and long years average

| Location | Mouths | Rainfall (mm) | | | Mean air temperature ($^0$C) | | | Mean relative air humidity (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Long term | 2005-2006 | 2006-2007 | Long term | 2005-2006 | 2006-2007 | Long term | 2005-2006 | 2006-2007 |
| Konya | October | 32.4 | 34.7 | 66.1 | 12.4 | 10.6 | 13.4 | 59.0 | 71.5 | 68.8 |
| | November | 36.1 | 68.8 | 51.9 | 5.7 | 4.9 | 4.7 | 70.0 | 76.4 | 74.8 |
| | December | 41.4 | 9.8 | 0.1 | 1.4 | 1.5 | -0.2 | 77.0 | 76.6 | 71.8 |
| | January | 34.8 | 21.2 | 20.9 | -0.3 | -2.9 | 2.0 | 76.0 | 80.2 | 64.0 |
| | February | 24.1 | 23.8 | 19.3 | 1.0 | 1.2 | 0.3 | 70.0 | 77.2 | 74.0 |
| | March | 26.5 | 18.4 | 15.4 | 5.4 | 7.1 | 5.7 | 62.0 | 70.2 | 53.0 |
| | April | 39.5 | 53.4 | 16.1 | 10.9 | 12.2 | 9.2 | 58.0 | 61.6 | 57.0 |
| | May | 43.5 | 17.9 | 16.3 | 15.6 | 16.2 | 20.6 | 55.0 | 59.2 | 41.0 |
| | June | 21.9 | 9.9 | 15.9 | 20.1 | 22.0 | 23.3 | 47.0 | 43.4 | 39.0 |
| | July | 7.9 | 0.3 | 0.4 | 23.5 | 23.2 | 26.2 | 42.0 | 45.1 | 28.0 |
| | August | 5.5 | 0.0 | 6.0 | 22.9 | 26.8 | 26.3 | 43.0 | 39.9 | 35.0 |
| | September | 10.0 | 20.0 | 4.1 | 18.6 | 18.2 | 21.3 | 46.0 | 55.0 | 32.0 |
| | Mean | - | - | - | 11.4 | 11.8 | 12.5 | 58.0 | 63.0 | 53.2 |
| | Total | 323.6 | 278.2 | 227.5 | | | | | | |
| Ankara | October | 26.0 | 28.0 | 37.1 | 12.8 | 10.8 | 13.6 | 58.0 | 66.0 | 70.2 |
| | November | 32.1 | 48.1 | 19.0 | 7.0 | 6.1 | 5.6 | 70.0 | 69.3 | 71.2 |
| | December | 45.9 | 14.4 | 1.3 | 2.4 | 3.0 | 1.1 | 78.0 | 69.8 | 62.8 |
| | January | 39.0 | 35.5 | 39.0 | 0.0 | -1.7 | 1.3 | 76.0 | 73.2 | 76.0 |
| | February | 35.5 | 67.2 | 16.4 | 1.5 | 0.4 | 2.6 | 73.0 | 78.9 | 68.0 |
| | March | 36.8 | 40.4 | 37.5 | 5.6 | 7.5 | 7.3 | 65.0 | 64.0 | 59.0 |
| | April | 43.9 | 29.4 | 23.8 | 11.1 | 13.1 | 9.1 | 59.0 | 55.1 | 53.0 |
| | May | 52.0 | 29.5 | 17.9 | 15.8 | 16.6 | 20.4 | 58.0 | 57.8 | 41.0 |
| | June | 34.2 | 31.8 | 31.7 | 19.8 | 21.6 | 22.5 | 52.0 | 53.1 | 39.0 |
| | July | 15.1 | 2.2 | 3.9 | 23.2 | 23. | 26.7 | 45.0 | 49.2 | 29.0 |
| | August | 11.3 | 0.1 | 9.8 | 23.0 | 27.2 | 26.3 | 44.0 | 44.7 | 37.0 |
| | September | 17.3 | 78.3 | 0.0 | 18.5 | 18.2 | 20.7 | 48.0 | 58.0 | 35.0 |
| | Mean | - | - | - | 11.7 | 12.2 | 13.1 | 60.5 | 61.6 | 53.4 |
| | Total | 389.1 | 404.9 | 237.4 | - | - | - | - | - | - |

80 kg ha$^{-1}$of phosphorus in the second and third years. In both the locations, irrigation was applied two times per cutting, before and after cutting, by a sprinkler irrigation method for two years. When the plant is in the 1/10 bloom stage, the plants were harvested on May 24, July 10, August 18, September 25 in 2006, May 23, July 02, August 08, September 10 in 2007 in Konya; May 21, July 08, August 16, September 24 in 2006, May 20, July 01, August 05, September 09 in 2007 in Ankara. The total yield of four cuttings was used for analysis of protein and macro- and microelements.

After harvest, samples of alfalfa cultivars were prepared for each plot. The samples were dried in a forced oven at 70°C to constant dry weight to determine the moisture content (Aydin & Tosun 1991).

### DETERMINATION OF PROTEIN CONTENT

The protein content of the samples was determined by the Kjeldahl method using a Kjeldahl device (AACC 1990). A crude protein ratio was calculated by multiplying the nitrogen concentration by 6.25 (Kacar 1972).

### DETERMINATION OF MINERAL CONTENTS

About 0.5 g of a dried and ground sample was put into a burning cup and 2 mL of 35% $H_2O_2$ and 5 mL of 65% $HNO_3$ were added. The sample was incinerated in a MARS 5 microwave oven at 180°C and the residue was dissolved in a certain volume of distilled water (Acar et al. 2012; Ozcan 2004). Mineral concentrations were determined by ICP-OES (PerkinElmer Optima DV 2000), as per instructions of the manufacturer. The values obtained are being expressed as %, g kg$^{-1}$ or mg kg$^{-1}$of dry matter.

### STATISTICAL ANALYSIS

The data obtained were analyzed by a randomized complete block model ANOVA. The significance levels ranged $p$ = 0.05 – 0.01 for statistical analyses. Differences between means were assessed with the Least Significant Difference (LSD) test at $p$ = 0.01 or 0.05 levels of significance. Statistical analyses were performed using the MSTAT-C statistical software package.

## Results and Discussion

In this research, six alfalfa (*M. sativa* L.) cultivars were studied in two different locations for two years. The results obtained by the variance analysis for all characteristics studied are summarized in Table 3. The results showed that the protein and mineral contents of alfalfa cultivars ranged widely with regard to the most study parameters.

### Protein content

The results of the variance analysis showed that the protein content in alfalfa hay was significantly affected by the location ($p<0.05$) and by the cultivar ($p<0.01$), as well as by such interactions as year × cultivar ($p<0.01$) and location × year × cultivar ($p<0.01$). However, no statistically significant differences were found for the year and location × year and location × cultivar interactions (Table 3).

The mean protein content was greater in Ankara province (19.36%) than in Konya (18.48%). The protein contents of alfalfa cultivars were found to vary between 17.99% for cv2 and 19.52% for cv6. In terms of the year × cultivar interaction, the protein content varied between 17.25% for cv2 in 2006 and 19.75% for cv6 in 2007. For the year × location × cultivar interaction, the highest protein content (20.71%) was obtained for cv4 in Ankara in 2007, while the lowest value (16.07%) was found for cv2 in Konya in 2006 (Table 4).

Akca Pelen et al. (2013) registered protein contents of 13 to 20.4% for 32 alfalfa cultivars from 2002 to 2012. Karadag et al. (2011) found that protein contents were different among alfalfa cultivars and varied between 14.36 and 17.78%. Sabancı et al. (2013) mentioned that protein contents of alfalfa cultivars differed depending on the year and ranged 16.79 to 19.49% for different years. The findings of this study were similar to the above results. Smith (1969) showed that alfalfa grown in a warm regime (32°C day/24°C night) had a higher protein content than that grown in a cool regime (18°C day/10°C night). Protein contents of alfalfa varied depending on agricultural practices such as a harvesting stage (Collins & Taylor 1980), soil type, stage of growth (Wedin et al. 1956), inter-row space, fertilization (Haby et al. 1999), the temperature (Smith 1969) and also dormancy groups of alfalfa cultivars. In this study, the protein content differed depending on the location, cultivar, year × cultivar and location × year × cultivar interactions.

### Phosphorus (P) content

In this study, we found that the differences in the P content of alfalfa hay were statistically highly significant ($p<0.01$) between the locations, years, cultivars and location × year, location × cultivar, year × cultivar and location × year × cultivar interactions (Table 3).

The P content was greater for Ankara (2.98 g $kg^{-1}$) than for Konya (2.40 g $kg^{-1}$). The P content in 2006 was 2.40 g $kg^{-1}$, but in 2007 it was 2.98 g $kg^{-1}$. Among the cultivars, the highest P content (2.98 g $kg^{-1}$) was found in cv6 and the lowest P content (2.45 g $kg^{-1}$) was found in cv4. In terms of the location × year interaction, the P content ranged 2.28 g $kg^{-1}$ in Konya in 2006 to 3.43 g $kg^{-1}$ in Ankara in 2007. The location × cultivar interaction affected the P content, which varied from 2.29 g $kg^{-1}$for cv1 in Konya to 3.39 g $kg^{-1}$ for cv6 in Ankara. Considering the year × cultivar interaction, the P content ranged 2.20 g $kg^{-1}$ for cv2 in 2006 to 3.32 g $kg^{-1}$ for cv6 in 2007. Lastly, for the location × year × cultivar interaction the lowest P content (2.06 g $kg^{-1}$) was obtained for cv4 in Konya during 2006 growing season, while the highest P content (3.95 g $kg^{-1}$) was obtained for cv6 in Ankara during 2007 growing season (Table 4).

Collins and Taylor (1980) mentioned that, depending on the soil type P and lime in soil, the P content in alfalfa hay ranged 0.23 to 0.39%. In a study conducted by Collins and Taylor (1980) determining the influence of temperature on the yield and a chemical composition of Vernal alfalfa, it was found that the P content varied from 0.24 to 0.34%. Kacar (1972) mentioned that under normal agricultural conditions the amount of P in alfalfa varied less widely than the amounts of most other elements. It is rather unusual to obtain P values outside the range of 0.2 to 0.5%, although very low concentrations can occur in alfalfa growing on very deficient soils (Kacar 1972). The P content range for alfalfa hay obtained in this study is in agreement with the values reported earlier.

### Potassium (K) content

The results of the statistical analysis showed highly significant differences ($p<0.01$) between the years (Table 3). The potassium content was 15.42 g $kg^{-1}$ in 2006 and 19.10 g $kg^{-1}$ in 2007 (Table 4). Also, the effect of the location on the potassium content was significant ($p<0.01$). The highest potassium content (17.77 g $kg^{-1}$) was obtained in the Ankara province. With regard to the year × location interaction ($p<0.01$), the highest potassium content (21.74 g $kg^{-1}$) was found in Ankara in 2007. There were statistically significant differences ($p<0.01$) in the potassium content between the cultivars. cv6 gave the highest (17.78 g $kg^{-1}$) potassium content, while the lowest value (16.17 g $kg^{-1}$) was obtained for cv4. The effect of the year × cultivar interaction on the K content in alfalfa hay was highly significant ($p<0.01$), with the values ranging 14.56 g $kg^{-1}$ (cv4 in 2006) to 19.95 g $kg^{-1}$ (cv5 in 2007). The K contents in alfalfa hay were significantly affected by the location × year × cultivar interaction ($p<0.01$) and the highest K content (23.25 g $kg^{-1}$) was obtained for cv5 in Ankara during 2007 growing season. Turan et al. (2010) indicated that boron application increased tissue K accumulation and the K content ranged 16 to 38 g $kg^{-1}$. Tongel and Ayan (2010) mentioned that the K content of alfalfa varied from 2.96 to 5.83%. Scholtz et al. (2009) found the K content to vary between 10.6 and 42.7 g $kg^{-1}$ in 168 South African alfalfa hay samples. Kacar (1972) indicated that over a 2-year period K values ranged 1.98 to 3.23%. When compared with other published data on the K content of alfalfa hay, the results of the present study were generally similar. The differences could be partly explained by genotypic differences, the vegetative parts used, a stage of maturity, levels of available Cu in the soil and soil pH (Khan et al. 2006). Animals need K for milk production, body fluid retention, nerve impulse transmission, muscle contraction and the maintenance of enzyme systems (Lemus 2013) and the recommended levels of K for all forages are over 8 g $kg^{-1}$ for grazing animals (Underwood 1981). Potassium is the most abundant mineral element in alfalfa and is usually present in a sufficient quantity for animals.

### MAGNESIUM (MG) CONTENT

The differences in the magnesium content were highly significant ($p$<0.01) between the years (Table 3). The magnesium content was 2.06 g $kg^{-1}$ in 2006 and 1.88 g $kg^{-1}$ in 2007 (Table 4). The location effect on the magnesium content was also highly significant ($p$<0.01). The highest magnesium content (2.08 g $kg^{-1}$) was obtained for Konya. The effect of the cultivars on the magnesium content was highly significant ($p$<0.01). The magnesium content was higher in CV5 (2.16 g $kg^{-1}$) than in the other cultivars. Also, the effects of the location × year, location × cultivar, year × cultivar and location × year × cultivar interactions were highly significant ($p$<0.01). The highest Mg contents for the location × year, location × cultivar, year × cultivar, and location × year × cultivar interactions were found to be 2.22 g $kg^{-1}$ (Konya 2007), 2.20 g $kg^{-1}$ (Konya for CV5), 2.32 g $kg^{-1}$ (2006 for CV5) and 2.53 g $kg^{-1}$ (Ankara × 2006 × CV5), respectively.

While these results were in agreement with the earlier reports by Allen et al. (1989), Nancy (1992), Tongel and Ayan (2010) and Turan et al. (2010), the values were lower than those obtained by Collins (1989), Jenking and Bottemley (1984) and Smith (1969). The differences in the content of Mg found in this study, compared with the literature data, could partly be explained by differences between forage species, levels of Mg in the soil, influences of the location and climate, a growth stage, the proportion of leaf and stem fractions collected for mineral analysis, and the season when forage sampling was performed (Khan et al. 2009, 2006).

Animals need Mg for skeletal growth, milk production, nerve impulse transmission, muscular control and the maintenance of enzyme systems (Lemus 2013). Herbage Mg concentrations are usually within the range of 0.08 to 0.30%, and legumes generally have a higher concentration of Mg than grasses (Kacar 1972).

### CALCIUM (CA) CONTENT

It was observed that the effects of the year, location, cultivar and year × location, location × cultivar, year × cultivar and location × year × cultivar interactions on the Ca content were highly significant ($p$<0.01) (Table 3). The Ca content was 16.04 g $kg^{-1}$ in 2006 and 12.56 g $kg^{-1}$ in 2007 (Table 5). In general, the Ca contents in Konya and Ankara provinces were found to be comparable; however, the Ca content in Konya (15.91 g $kg^{-1}$) was higher. When comparing the effects of the location × year interaction, the highest Ca content (16.51 g $kg^{-1}$) was obtained for Ankara in 2006. Among the cultivars, CV4 had the highest Ca content (16.39 g $kg^{-1}$). The highest Ca contents for the location × cultivar and year × cultivar interactions were obtained for CV4 in Konya (15.55 g $kg^{-1}$) and CV5 in 2006 (19.41 g $kg^{-1}$), respectively. The Ca contents for the location × year × cultivar interaction ranged 6.71 (Ankara × 2007 × CV1) to 22.81 g $kg^{-1}$ (Ankara × 2006 × CV5). Similar results were reported by Tongel and Ayan (2010) who found that Ca contents of alfalfa hay ranged 1.08 to 2.33%. Smith (1970) in a study on the influence of temperature on the chemical composition mentioned that Ca concentrations in alfalfa varied from 0.97 to 1.26%. In another study, Ca concentrations ranged 1.55 to 1.89% in a cool/warm regime and a cool regime, respectively (Smith 1969).

Animals need Ca for skeletal growth, milk production, nerve impulse transmission, and the maintenance of enzyme systems (Lemus 2013). Forage Ca requirements of grazing ruminants are a subject of considerable debate as the requirement is influenced by the animal type, level of production, age and weight (Khan et al. 2006). A forage for ruminants should contain at least 0.3% Ca (Kidambi et al. 1989; Tejada et al. 1985). The alfalfa Ca values found in this study were sufficiently higher than the ruminant requirements and thus may be considered adequate for

TABLE 3. Analysis of variance with main effect and interaction effect of different factors (six alfalfa cultivars; two locations and two years) on protein and mineral contents

| Source | PC | *P* | *K* | Mg | Ca | Na | Mn | Fe | Zn | B | Mo | Cu | Cr | Se | Al |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location (L) | * | ** | ** | ** | ** | ** | * | ns | * | * | ** | * | ns | ns | ns |
| Year (Y) | ns | ** | ** | ** | ** | ** | ** | ** | ** | ns | ** | ns | * | * | ns |
| L x Y | ns | ** | ** | ** | ** | ** | ** | ns | ** | ns | ** | ns | * | ns | * |
| Genotype (G) | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | * | ns |
| L x G | ns | ** | ns | ** | ** | ** | ** | ** | ** | ** | ** | ns | ** | ns | ** |
| Y xG | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | * |
| L x Y x G | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ns | ** | ** | * |

F-test significance: *: P ≤ 0.05 ; **: P ≤ 0.01 ; ns: not significant optimum performance of ruminants. Similar conclusions were also made by other researchers such as Khan et al. (2006) and Tongel and Ayan (2010).

### SODIUM (NA) CONTENT

The results of the analysis of variance showed that the Na contents in alfalfa were significantly affected by the location, year, cultivar and location × year, location × cultivar, year × cultivar, and location × year × cultivar interactions ($p$<0.01) (Table 3).

The Na content was 1.44 g kg$^{-1}$ in 2006 and 2.66 g kg$^{-1}$ in 2007 and was higher in Ankara than in Konya (Table 5). For the location × year interaction, the highest Na content (3.96 g kg$^{-1}$) was obtained for Ankara during 2007 growing season. The Na contents of the alfalfa cultivars ranged from 1.20 g kg$^{-1}$ in CV5 to 2.78 g kg$^{-1}$ in CV3. The highest Na contents for the location × cultivar and year × cultivar interactions were obtained for CV6 in Ankara (3.89 g kg$^{-1}$) and CV3 in 2007 (3.68 g kg$^{-1}$), respectively. The Na contents for the location × year × cultivar interaction ranged 0.96 g kg$^{-1}$(Ankara × 2006 × CV1) to 5.65 g kg$^{-1}$ (Ankara × 2007 × CV3).

Similar results were obtained by Walzl et al. (2011) who mentioned that Na contents in alfalfa ranged from 0.9 to 1.0 g kg$^{-1}$. Turan et al. (2010) found that Na contents in different locations and at different boron application rates ranged in alfalfa from 880 to 1,090 mg kg$^{-1}$. Kacar (1972) mentioned that herbage Na concentrations varied more than a thousand-fold, from 0.002 to 2.12% and any values between 0.05 and 1% would not be considered unusual. Animals need Na for glucose and amino acid transport, for retaining body fluids and maintaining acid-base balance (Lemus 2013). Khan et al. (2009) mentioned that, as quoted by other researchers, Na requirements for ruminants are debatable, yet the range from 1–4 g kg$^{-1}$is recommended. A dairy Holstein cow with milk production of 35 kg day$^{-1}$ needs 2.3 g of Na kg$^{-1}$of dry matter herbage (NRC 2001). According to the results of the present study, the Na concentrations were low or equivalent to the levels recommended for optimal animal production.

### MICROELEMENTS

The variance analysis showed statistically highly significant effects of the locations on the Mo contents in alfalfa hay (Table 3). The location effects were also statistically significant on the Mn, Zn, B and Cu contents. However, locations had no effects on the Fe, Cr, Se and Al contents. The year effects were highly significant on Mn, Fe, Zn and Mo and significant on Cr and Se, but there were no effects on the B, Cu and Al contents. The location × year interaction had statistically highly significant effects on Mn, Zn and Mo, significant effects on Cr and Al, but no effects on the Fe, B, Cu, and Se contents. The cultivars had statistically highly significant effects on the contents of all microelements, except Al. The location × cultivar interaction had statistically highly significant effects on the contents of all microelements, except Cu and Se. The year × cultivar interaction had highly significant effects on the contents of all microelements, except Al, which was affected significantly. Lastly, the location × year × cultivar interaction had highly significant effects on Mn, Fe, Zn, B, Mo, Cr and Se and a significant effect on Al, but no effect on the Cu content in alfalfa hay (Table 3).

The manganese (Mn) contents of alfalfa hay ranged 15.4 to 54.3 mg kg$^{-1}$, with an average of 34.60 mg kg$^{-1}$ (Table 5). Similar results on Mn contents of alfalfa were reported by Walzl et al. (2011) (43.5 to 47.7 mg kg$^{-1}$), Turan et al. (2010) (8.0 to 33.0 mg kg$^{-1}$) and Tongel and Ayan (2010) (13.10-39.54 mg kg$^{-1}$). A dairy Holstein cow with milk production of 35 kg day$^{-1}$ needs 15.0 mg of Mn kg$^{-1}$of dry matter herbage (NRC 2001).

The iron (Fe) contents in alfalfa hay ranged 44.3 to 92.7 mg kg$^{-1}$, with an average of 69.7 mg kg$^{-1}$ (Table 5). Almost similar results were reported by Walzl et al. (2011) who found that Fe contents in alfalfa hay ranged from 63.1 to 69.8 mg kg$^{-1}$. However, Turan et al. (2010) found that Fe contents of alfalfa varied from 62 to 188 mg kg$^{-1}$, which is higher than the results of this study. Even higher results were reported by Tongel and Ayan (2010) who mentioned that Fe contents of alfalfa were between 209.3 and 343.1 mg kg$^{-1}$ and by Scholtz et al. (2009) who noted that Fe contents in alfalfa ranged 149 to 3,138 mg kg$^{-1}$. According to Kacar (1972), soil pH is the most important factor governing Fe uptake. Also, deficiency of Fe is due to low availability of insoluble oxides and phosphates and therefore, is most likely to occur on calcareous soils. Other researchers mentioned that differences in Fe contents could be partly explained by variations in the content of Fe in soils and in climatic conditions between locations (Khan et al. 2006). The zinc (Zn) contents in alfalfa hay ranged from 24.6 to 121.9 mg kg$^{-1}$, with an average of 56.4 mg kg$^{-1}$ (Table 6). These findings were similar to the ranges reported by other researchers for alfalfa cultivars, 24.89-83.01 mg kg$^{-1}$ (Tongel & Ayan 2010), 25-85 mg kg$^{-1}$ (Turan et al. 2010) and 23-75 mg kg$^{-1}$ (Scholtz et al. 2009). However, Walzl et al. (2010) found that the Zn content of alfalfa varied from 22.9 to 25.0 mg kg$^{-1}$, which is lower than the results of this study. A dairy Holstein cow with milk production of 35 kg day$^{-1}$ needs 14 mg of Zn kg$^{-1}$of dry matter herbage (NRC 2001). Dietary requirements of ruminants for Zn ranged 12 to 30 mg kg$^{-1}$ (Khan et al. 2009). Thus, the alfalfa Zn values found in this study were considered adequate for ruminants.

The boron (B) contents in alfalfa hay ranged from 46.4 to 85.4 mg kg$^{-1}$, with an average of 65.4 mg kg$^{-1}$ (Table 6). The B values in this study were higher than the 18.8-19.6 mg kg$^{-1}$ range reported by Walzl et al. (2011), but similar to the 30-52 mg kg$^{-1}$ range reported by Smith (1969) and the 37-52 mg kg$^{-1}$ range reported by Caldwell et al. (1969). The molybdenum (Mo) contents in alfalfa hay ranged from 0.50 to 6.13 mg kg$^{-1}$, with an average of 1.94 mg kg$^{-1}$ (Table 6). These results agree with earlier reports by Walzl et al. (2011) who found that Mo contents

TABLE 4. Protein (%), P, K and Mg content (g $kg^{-1}$) of Alfalfa cultivars in different locations and years

| Year | Cultivars | Protein content (%) | | | P content (g $kg^{-1}$) | | | K content (g $kg^{-1}$) | | | Mg content (g $kg^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Locations | | | Locations | | | Locations | | | Locations | | |
| | | Konya | Ankara | Mean | Konya | Ankara | Mean | Konya | Ankara | Mean | Konya | Ankara | Mean |
| 2006 | CV1 | 18.55 b-g | 19.44 a-e | 19.00 ab | 2.17 i-k | 2.40. gh | 2.28 e | 16.84 e-h | 13.74 k | 15.29 cd | 1.76 hi | 2.26 bc | 2.01 cd |
| | CV2 | 16.07 h | 18.42 d-g | 17.25 d | 2.13 jk | 2.26 h-k | 2.20 e | 15.43 h-j | 13.70 k | 14.56 d | 1.83 g-i | 1.98 e-g | 1.91 de |
| | CV3 | 19.47 a-e | 19.65 a-e | 19.56 ab | 2.45 gh | 2.46 gh | 2.46 d | 18.34 c-e | 13.96 i-k | 16.15 c | 2.22 b-d | 2.14 c-e | 2.18 b |
| | CV4 | 17.23 fgh | 18.09 efg | 17.66 cd | 2.06 k | 2.35 g-j | 2.21 e | 15.99 gh | 13.65 k | 14.82 d | 1.78 hi | 1.93 f-h | 1.85 e |
| | CV5 | 19.97 a-d | 19.40 a-e | 19.69 a | 2.39 gh | 2.80 de | 2.60 cd | 17.13 d-g | 13.85 jk | 15.49 cd | 2.12 c-f | 2.53 a | 2.32 a |
| | CV6 | 18.45 c-g | 20.13 abc | 19.29ab | 2.45 gh | 2.84 de | 2.65 c | 18.58 cd | 13.90 jk | 16.24 c | 1.99 e-g | 2.23 b-d | 2.11 bc |
| | Mean | 18.29 | 19.19 | 18.74 | 2.28 C | 2.52 B | 2.40 | 17.05 B | 13.80 D | 15.43 | 1.95 B | 2.18 A | 2.06 |
| 2007 | CV1 | 19.41 a-e | 18.69 b-f | 19.05 ab | 2.41 gh | 2.95 d | 2.68 c | 17.60 d-f | 21.17 b | 19.39 a | 2.05 d-f | 1.31 k | 1.68 f |
| | CV2 | 18.77 b-f | 18.69 b-f | 18.73 abc | 2.72 ef | 3.34 c | 3.03 b | 16.50 f-h | 21.80 ab | 19.15 a | 2.33 b | 1.34 k | 1.83 e |
| | CV3 | 16.85 gh | 19.86 a-d | 18.36 bcd | 2.37 g-i | 3.73 ab | 3.05 b | 15.87 gh | 22.61 ab | 19.24 a | 2.12 c-f | 1.55 j | 1.83 e |
| | CV4 | 18.32 d-g | 20.71 a | 19.52 ab | 2.41 gh | 3.00 d | 2.71 c | 15.51 hi | 19.53 c | 17.52 b | 2.27 bc | 1.64 ij | 1.95 de |
| | CV5 | 18.43 c-g | 19.91 a-d | 19.17 ab | 2.53 fg | 3.60 b | 3.07 b | 16.65 f-h | 23.25 a | 19.95 a | 2.28 bc | 1.72 ij | 2.00 cd |
| | CV6 | 20.18 ab | 19.33 a-e | 19.75 a | 2.69 ef | 3.95 a | 3.32 a | 16.59 f-h | 22.07 ab | 19.33 a | 2.26 bc | 1.75 hi | 2.01 cd |
| | Mean | 18.66 | 19.53 | 19.10 | 2.52 B | 3.43 A | 2.98 | 16.45 C | 21.74 A | 19.10 | 2.22 A | 1.55 C | 1.88 |
| Mean of years | CV1 | 18.98 | 19.07 | 19.02 ab | 2.29 gh | 2.68 cd | 2.48 d | 17.22 | 17.47 | 17.34 ab | 1.90 cd | 1.78 de | 1.84 c |
| | CV2 | 17.42 | 18.56 | 17.99 c | 2.43 efg | 2.80 c | 2.61 c | 15.97 | 17.75 | 16.86 bc | 2.08 ab | 1.66 e | 1.87 c |
| | CV3 | 18.16 | 19.76 | 18.96 ab | 2.41 fg | 3.10 b | 2.76 b | 17.11 | 18.29 | 17.70 a | 2.17 a | 1.84 d | 2.01 b |
| | CV4 | 17.77 | 19.40 | 18.59 bc | 2.24 h | 2.68 cd | 2.46 d | 15.75 | 16.59 | 16.17 c | 2.02 bc | 1.79 de | 1.90 c |
| | CV5 | 19.20 | 19.66 | 19.43 ab | 2.46 ef | 3.20 b | 2.83 b | 16.89 | 18.55 | 17.72 a | 2.20 a | 2.12 ab | 2.16 a |
| | CV6 | 19.32 | 19.73 | 19.52 a | 2.57 de | 3.39 a | 2.98 a | 17.59 | 17.98 | 17.78 a | 2.13 ab | 1.99 bc | 2.06 b |
| | Mean | 18.48 | 19.36 | 18.92 | 2.40 | 2.98 | 2.69 | 17.25 | 17.77 | 17.26 | 2.08 | 1.86 | 1.97 |

TABLE 5. Ca and Na (g kg$^{-1}$), Mn and Fe content (mg kg$^{-1}$) of Alfalfa cultivars in different locations and years

| Year | Cultivars | Ca content (g kg$^{-1}$) | | | Na content (g kg$^{-1}$) | | | Mn content (mg kg$^{-1}$) | | | Fe content (mg kg$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Locations | | | Locations | | | Locations | | | Locations | | |
| | | Konya | Ankara | Mean | Konya | Ankara | Mean | Konya | Ankara | Mean | Konya | Ankara | Mean |
| 2006 | CV1 | 13.43 h | 16.86 b-e | 15.15 bcd | 1.29 kl | 0.96 op | 1.12 g | 33.3 e-h | 42.6 b-e | 38.0 bc | 69.3 d-g | 77.5 a-e | 73.4 abc |
| | CV2 | 15.52 d-h | 14.67 e-h | 15.10 cd | 1.46 ij | 1.40 jk | 1.43 e | 38.3 c-f | 40.7 b-e | 39.5 b | 83.8 a-d | 58.6 gh | 71.2 a-d |
| | CV3 | 16.33 b-f | 14.65 e-h | 15.49 bcd | 1.80 g | 1.95 f | 1.86 d | 43.5 b- | 30.6 f-h | 37.1 bcd | 86.7 a-c | 68.0 d-g | 77.3 ab |
| | CV4 | 17.17 b-d | 16.36 b-f | 16.76 b | 1.44 ij | 1.09 m-o | 1.26 f | 30.9 f-h | 38.5 b-f | 34.7 b-f | 59.1 gh | 75.4 b-f | 67.3 b-e |
| | CV5 | 16.00 c-d | 22.81 a | 19.41 a | 1.15 l-n | 0.91 p | 1.03 g | 41.5 b-e | 54.3 a | 47.9 a | 75.5 b-f | 88.2 ab | 81.8 a |
| | CV6 | 14.96 d-h | 13.73 gh | 14.34 d | 1.70 gh | 2.15 e | 1.92 d | 36.4 c-g | 34.3 d-h | 35.4 b-f | 80.5 a-d | 64.0 e-g | 72.3 a-d |
| | Mean | 15.57 A | 16.51 A | 16.04 | 1.47 B | 1.41 B | 1.44 | 37.3 A | 40.2 A | 38.8 | 75.8 | 71.9 | 73.9 |
| 2007 | CV1 | 15.63 d-h | 6.71 j | 11.17 ef | 1.12 mn | 4.68 b | 2.90 b | 36.3 c-g | 17.2 jk | 26.7 g | 60.6 fg | 68.9 d-g | 64.7 cde |
| | CV2 | 18.57 b | 6.71 j | 12.64 e | 1.51 ij | 3.61 c | 2.56 c | 47.9 ab | 15.4 k | 31.7 c-g | 71.5 c-g | 44.3 h | 57.9 e |
| | CV3 | 14.37 f-h | 6.72 j | 10.55 f | 1.72 g | 5.65 a | 3.68 a | 39.0 b-f | 18.5 i-k | 28.8 fg | 70.9 c-g | 92.7 a | 81.8 a |
| | CV4 | 17.94 bc | 14.09 f-h | 16.02 bc | 1.20 lm | 2.49 d | 1.84 d | 44.3 bc | 27.2 g-i | 35.8 b-e | 79.3 a-e | 57.3 gh | 68.3 b-e |
| | CV5 | 14.79 e-h | 10.02 i | 12.41 e | 1.05 n-p | 1.71 g | 1.38 e | 35.7 c-g | 25.2 h-j | 30.5 d-g | 56.6 gh | 61.1 fg | 58.8 e |
| | CV6 | 16.17 c-f | 9.00 i-j | 12.59 e | 1.56 hi | 5.63 a | 3.59 a | 37.8 c-f | 20.6 i-k | 29.2 efg | 60.3 f-h | 63.4 e-g | 61.8 e |
| | Mean | 16.25 A | 8.88 B | 12.56 | 1.36 B | 3.96 A | 2.66 | 40.2 A | 20.7 B | 30.4 | 66.5 | 646 | 65.6 |
| Mean of years | CV1 | 14.53 d | 11.75 e | 13.16 b | 1.20 h | 2.82 b | 2.01 b | 34.8 bcd | 29.9 def | 17.2 jk | 64.9 c | 7.32 abc | 69.1 b |
| | CV2 | 17.04 ab | 10.69 e | 13.87 b | 1.49 f | 2.50 c | 1.99 b | 43.1 a | 28.1 def | 15.4 k | 77.7 ab | 51.4 d | 64.6 b |
| | CV3 | 15.35 cd | 10.69 e | 13.02 b | 1.76 d | 3.80 a | 2.78 a | 41.3 ab | 24.6 f | 18.5 i-k | 78.8 a | 80.3 a | 79.6 a |
| | CV4 | 17.55 a | 15.23 cd | 16.39 a | 1.32 g | 1.79 d | 1.55 c | 37.6 abc | 32.9 cde | 27.2 g-i | 69.2 abc | 66.3 bc | 67.8 b |
| | CV5 | 15.40 cd | 16.42 abc | 15.91 a | 1.10 i | 1.31 g | 1.20 d | 38.6 abc | 39.8 ab | 25.2 h-j | 66.0 c | 74.6 abc | 70.3 b |
| | CV6 | 15.56 bcd | 11.36 e | 13.46 b | 1.63 e | 3.89 a | 2.76 a | 37.1 abc | 27.5 ef | 20.6 i-k | 70.4 abc | 63.7 c | 67.0 b |
| | Mean | 15.91 | 12.69 | 14.30 | 1.42 | 2.68 | 2.05 | 38.7 | 30.4 | 34.6 | 71.7 | 68.8 | 69.7 |

TABLE 6. Zn, B, Mo and Cu content (mg kg$^{-1}$) of Alfalfa cultivars in different locations and years

| Year | Cultivars | Zn content (mg kg$^{-1}$) | | | B content (mg kg$^{-1}$) | | | Mo content (mg kg$^{-1}$) | | | Cu content (mg kg$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Locations | | | Locations | | | Locations | | | Locations | | |
| | | Konya | Ankara | Mean | Konya | Ankara | Mean | Konya | Ankara | Mean | Konya | Ankara | Mean |
| 2006 | CV1 | 73.2 de | 49.6 g-i | 61.4 d | 53.7 hi | 65.2 d-g | 59.5 ef | 0.50 k | 0.83 hij | 0.67 i | 4.00 | 4.10 | 4.05 a |
| | CV2 | 91.7 c | 52.8 gh | 72.2 b | 72.2 b-d | 71.6 b-d | 71.9 bc | 0.53 k | 0.70 ijk | 0.62 i | 3.90 | 3.63 | 3.77 d |
| | CV3 | 103.7 b | 43.7 h-j | 73.7 b | 83.4 a | 54.9 g-i | 69.2 bcd | 0.70 ijk | 1.37 f | 1.03 g | 4.17 | 3.80 | 3.98 abc |
| | CV4 | 74.7 de | 65.6 ef | 70.2 bc | 56.5 g-i | 67.5 d-f | 62.0 def | 0.60 k | 0.67 jk | 0.63 i | 3.87 | 3.90 | 3.88 bcd |
| | CV5 | 121.9 a | 60.4 fg | 91.2 a | 85.4 a | 79.9 ab | 82.6 a | 0.50 k | 1.20 fg | 0.85 h | 3.87 | 3.87 | 3.87 cd |
| | CV6 | 84.3 cd | 43.2 h-j | 63.7 cd | 60.5 e-f | 52.7 hi | 56.6 ef | 0.70 ijk | 2.00 e | 1.35 f | 3.97 | 3.60 | 3.78 d |
| | Mean | 91.6 A | 52.6 B | 72.1 | 68.6 | 65.3 | 67.0 | 0.59 D | 1.13 B | 0.86 | 3.96 | 3.82 | 3.89 |
| 2007 | CV1 | 43.4 h-j | 29.9 kl | 36.7 f | 64.3 d-g | 53.1 hi | 58.7 ef | 0.90 hi | 3.80 d | 2.35 e | 4.07 | 3.60 | 3.83 d |
| | CV2 | 53.4 gh | 24.6 l | 39.0 f | 79.0 a-c | 46.4 i | 62.7 def | 0.93 h | 5.93 a | 3.43 a | 3.90 | 3.27 | 3.58 e |
| | CV3 | 50.0 g-i | 26.1 l | 38.1 f | 65.0 d-g | 69.0 c-d | 67.0 cd | 0.83 hij | 5.13 b | 2.98 c | 3.53 | 3.13 | 3.33 f |
| | CV4 | 51.8 gh | 46.6 h-j | 49.2 e | 84.0 a | 68.5 de | 76.2 ab | 1.00 gh | 5.33 b | 3.17 b | 4.07 | 3.97 | 4.02 ab |
| | CV5 | 42.9 h-j | 38.6 i-k | 40.7 f | 64.8 d-g | 61.8 d-h | 63.3 de | 0.93 h | 4.40 c | 2.67 d | 4.30 | 3.87 | 4.08 a |
| | CV6 | 46.2 h-j | 35.0 j-k | 40.6 f | 77.4 f-h | 53.5 hi | 55.4 f | 0.90 hi | 6.13 a | 3.52 a | 3.80 | 3.80 | 3.80 d |
| | Mean | 48.0 B | 33.5 C | 40.7 | 69.1 | 58.7 | 63.9 | 0.92 C | 5.12 A | 3.02 | 3.94 | 3.61 | 3.77 |
| Mean of years | CV1 | 58.3 de | 39.7 g | 49.0 d | 59.0 de | 59.2 de | 59.1 c | 0.70 f | 2.32 e | 1.51 e | 4.03 | 3.85 | 3.94 a |
| | CV2 | 72.5 bc | 38.7 g | 55.6 bc | 75.6 a | 59.0 de | 67.3 b | 0.73 f | 3.32 b | 2.03 b | 3.90 | 3.45 | 3.67 c |
| | CV3 | 76.9 ab | 34.9 g | 55.9 bc | 74.2 ab | 61.9 cd | 68.1 ab | 0.77 f | 3.25 b | 2.01 bc | 3.85 | 3.47 | 3.66 c |
| | CV4 | 63.3 de | 56.1 ef | 59.7 b | 70.2 ab | 68.0 bc | 69.1 ab | 0.80 f | 3.00 c | 1.90 c | 3.97 | 3.93 | 3.95 a |
| | CV5 | 82.4 a | 49.5 f | 65.9 a | 75.1 ab | 70.8 ab | 73.0 a | 0.72 f | 2.80 d | 1.76 d | 4.08 | 3.87 | 3.97 a |
| | CV6 | 65.3 cd | 39.1 g | 52.2 cd | 58.9 de | 53.1 e | 56.0 c | 0.80 f | 4.07 a | 2.43 a | 3.88 | 3.70 | 3.79 b |
| | Mean | 69.8 | 43.0 | 56.4 | 68.8 | 62.0 | 65.4 | 0.75 | 3.12 | 1.94 | 3.95 | 3.71 | 3.83 |

TABLE 7. Cr, Se and Al content (mg $kg^{-1}$) of Alfalfa cultivars in different locations and years

| Year | Cultivars | Cr content (mg $kg^{-1}$) | | | Se content (mg $kg^{-1}$) | | | Al content (mg $kg^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Locations | | | Locations | | | Locations | | |
| | | Konya | Ankara | Mean | Konya | Ankara | Mean | Konya | Ankara | Mean |
| 2006 | CV1 | 1.17 b-e | 0.93 f | 1.05 de | 1.03 a | 0.73 c-f | 0.88 a | 45.8 a-e | 33.2 e-g | 39.5 ab |
| | CV2 | 1.20 bcd | 1.17 b-e | 1.18 bcd | 0.77 cde | 0.73 c-f | 0.75 a-d | 57.2 ab | 36.2 e-g | 46.7 ab |
| | CV3 | 1.33 bc | 1.20 bcd | 1.27 b | 0.73 c-f | 1.00 ab | 0.87 ab | 52.1 a-d | 39.9 d-g | 46.0 ab |
| | CV4 | 1.20 bcd | 0.93 f | 1.07 cde | 0.87 abc | 0.60 d-h | 0.73 bcd | 36.7 e-g | 35.1 e-g | 35.9 b |
| | CV5 | 1.13 cdef | 1.20 bcd | 1.17 b-e | 0.73 c-f | 0.80 bcd | 0.77 abc | 42.1 c-g | 57.0 ab | 49.5 a |
| | CV6 | 1.03 def | 1.17 b-e | 1.10 cde | 0.57 e-h | 0.77 cde | 0.67 cde | 44.6 b-e | 33.5 e-g | 39.1 ab |
| | Mean | 1.18 B | 1.10 C | 1.14 | 0.78 | 0.77 | 0.78 | 46.4 A | 39.2 B | 42.8 |
| 2007 | CV1 | 1.10 def | 1.13 c-f | 1.12 cde | 0.53 fgh | 0.47 h | 0.50 f | 31.8 fg | 57.8 a | 44.8 ab |
| | CV2 | 1.20 bcd | 1.33 bc | 1.27 b | 0.57 e-h | 0.50 gh | 0.53 ef | 43.6 c-g | 38.6 e-g | 41.1 ab |
| | CV3 | 1.07 def | 2.40 a | 1.73 a | 0.87 abc | 0.60 d-h | 0.73 bcd | 39.3 e-g | 44.2 c-f | 41.7 ab |
| | CV4 | 1.10 def | 0.97 ef | 1.03 e | 0.53 fgh | 0.70 c-g | 0.62 def | 40.2 d-g | 38.8 e-g | 39.5 ab |
| | CV5 | 1.37 b | 1.03 def | 1.20 bc | 0.90 abc | 0.53 fgh | 0.72 cd | 31.4 g | 40.1 d-g | 35.7 b |
| | CV6 | 1.13 c-f | 0.97 ef | 1.05 de | 0.77 cde | 0.60 d-h | 0.68 cd | 53.0 a-c | 41.4 c-g | 47.2 ab |
| | Mean | 1.16 BC | 1.31 A | 1.23 | 0.69 | 0.57 | 0.63 | 39.9 B | 43.5 AB | 41.7 |
| Mean of years | CV1 | 1.13 bcd | 1.03 de | 1.08 cd | 0.78 | 0.60 | 0.69 b | 38.8 abc | 45.5 abc | 42.2 |
| | CV2 | 1.20 bc | 1.25 b | 1.22 b | 0.67 | 0.62 | 0.64 b | 50.4 a | 37.4 bc | 43.9 |
| | CV3 | 1.20 bc | 1.80 a | 1.50 a | 0.80 | 0.80 | 0.80 a | 45.7 abc | 42.1 abc | 43.9 |
| | CV4 | 1.15 bcd | 0.95 e | 1.05 d | 0.70 | 0.65 | 0.67 b | 38.5 abc | 37.0 bc | 37.7 |
| | CV5 | 1.25 b | 1.12 bcd | 1.18 bc | 0.82 | 0.67 | 0.74 ab | 36.7 c | 48.5 abc | 42.6 |
| | CV6 | 1.08 cde | 1.07 cde | 1.07 d | 0.67 | 0.68 | 0.67 b | 48.8 ab | 37.4 bc | 43.1 |
| | Mean | 1.17 | 1.20 | 1.19 | 0.74 | 0.67 | 0.70 | 43.1 | 41.3 | 42.2 |

in alfalfa ranged from 0.3 to 1.4 mg kg$^{-1}$ and by Caldwell et al. (1969) who found that Mo contents ranged 1.6-1.9 mg kg$^{-1}$. The Mo concentrations in 24 alfalfa samples from New Jersey farms ranged less than 0.1 to 1.44 mg kg$^{-1}$, with an average of 0.88 mg kg$^{-1}$ (Kacar 1972).

The copper (Cu) contents in alfalfa hay ranged from 3.13 to 4.17 mg kg$^{-1}$, with an average of 3.83 mg kg$^{-1}$ (Table 6). Similar results were obtained by Smith (1969) who found that Cu contents in the Vernal alfalfa herbage harvested at first flower, following growth under cool and warm temperatures, varied from 3.0 to 4.0 mg kg$^{-1}$. However, some previous studies have presented conflicting results. Thus, higher values were obtained by Turan et al. (2010) (5-20 mg kg$^{-1}$) and Tongel and Ayan (2010) (from 3.08 to 15.69 mg kg$^{-1}$). Also, Walzl et al. (2011) obtained Cu contents ranging from 5.8 to 7.4 mg kg$^{-1}$. The differences among Cu values reported earlier could be partly explained by genotypic differences, the vegetative parts used, a stage of maturity, levels of available Cu in the soil and soil pH (Khan et al. 2006). The chromium (Cr) contents in alfalfa hay ranged from 0.93 to 2.40 mg kg$^{-1}$, with an average of 1.19 mg kg$^{-1}$ (Table 7). Walzl et al. (2011) showed a Cr content of 0.2 mg kg$^{-1}$ in alfalfa, which is lower than our results. The selenium (Se) contents in alfalfa hay ranged 0.77 to 1.03 mg kg$^{-1}$, with an average of 0.70 mg kg$^{-1}$ (Table 7). However, Se is important in animal nutrition because its trace amounts can prevent muscular dystrophy, while higher levels can cause a disease called blind staggers or alkali disease (Kacar 1972). The aluminum (Al) contents in alfalfa hay ranged from 31.2 to 57.8 mg kg$^{-1}$, with an average of 42.2 mg kg$^{-1}$ (Table 7). This agrees with an earlier report by Smith (1969) who found that Al contents in alfalfa ranged from 41 to 49 mg kg$^{-1}$.

## Conclusion

We found significant differences among the cultivars with regard to the contents of protein and several mineral elements. The importance of the location × year × cultivar interaction demonstrated that an appropriate cultivar should be selected or each region. Furthermore, since the protein content varied depending on the year, we recommend that protein and mineral contents are analyzed every year to prepare an accurate ration for feeding livestock. The levels of mineral elements in alfalfa hay were sufficient for many livestock species. The high crude protein content and mineral element levels in alfalfa hay, meeting the needs of many livestock species, are important for organic livestock farmers. In particular, alfalfa hay with high protein and mineral contents can be sufficient, without any supplementary foods, when alfalfa is under a snow cover during winter in organic livestock husbandry enterprises. Due to the fact that the crude protein and mineral element contents differed among the cultivars, increasing those should be one of the main objectives in future alfalfa breeding programs, along with improving the hay yield.

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