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#### ARAŞTIRMA MAKALESİ

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# Change of Forage Yield and Quality Characteristics of White Clover (Trifolium repens L.) at Different Harvest Time

Ak Üçgülün (Trifolium repens L.) Farklı Hasat Zamanlarına Ait Verim ve Kalite Özelliklerinin Değişimi

# Erdal CACAN<sup>1\*</sup>, Selim OZDEMIR<sup>2</sup>, Kagan KOKTEN<sup>3</sup>, Ridvan UCAR<sup>4</sup>, Sam MOKHTARZADEH<sup>5</sup>, Muammer EKMEKCI<sup>6</sup>, Mehmet Ali KUTLU<sup>7</sup>

#### Abstract

This study was conducted to determine the yield and quality characteristics of white clover at different harvest time. The study was carried out according to the randomized blocks experimental design in 2021 and 2022, and the Rivendel variety of white clover was used as the plant material in the study. Four different harvest time of white clover, as early bloom, mid-bloom, full bloom, and after-bloom, were considered as the research subject. In the experiment, the plant height, green forage yield, dry matter yield, dry matter ratio, crude protein, crude protein yield, insoluble fiber in acid detergent (ADF), insoluble fiber in neutral detergent (NDF), relative feed value and phosphorus, potassium, calcium, and magnesium contents of white clover were investigated. It has been determined that the differences in all of these examined features in different harvest time of white clover were statistically significant. In the research, white clover reached the highest plant height (32.3 cm and 27.8 cm), green forage yield (38.367 kg ha<sup>-1</sup> and 52.080 kg ha<sup>-1</sup>), dry matter yield (10.707 kg ha<sup>-1</sup> and 13.424 kg ha<sup>-1</sup>), and crude protein yield (1987 kg ha<sup>-1</sup> and 2544 kg ha<sup>-1</sup>) in full bloom in both years. In 2021, the highest crude protein and relative feed value and the lowest ADF and NDF ratios were obtained during early bloom and mid-bloom, while in 2022, the highest values for these characteristics were obtained only from early bloom stages. It was determined that the lowest phosphorus and potassium contents and the highest calcium and magnesium contents were obtained from the after bloom stage in both years. As a result, since the highest yield values and average quality values are obtained from the full bloom stage, it has been concluded that it was more advantageous for the producer to harvest the white clover at this stage.

Keywords: Harvest stage, Hay, Crude protein, Relative feed value, Macro elements

<sup>&</sup>lt;sup>1</sup>\*Sorumlu Yazar/Corresponding Author: Erdal Cacan, Bingol University, Vocational School of Food, Agriculture and Livestock, Department of Crop and Animal Production, Bingol, Turkey. E-mail: ecacan@bingol.edu.tr (D) OrcID: 0000-0002-9469-2495

<sup>&</sup>lt;sup>2</sup>Selim Ozdemir, Bingol University, Vocational School of Food, Agriculture and Livestock, Department of Crop and Animal Production, Bingol, Turkey. E-mail: sozdemir@bingol.edu.tr <sup>[D]</sup> OrcID: 0000-0003-1840-9907

<sup>&</sup>lt;sup>3</sup>Kagan Kokten, Sivas University of Science and Technology, Faculty of Agricultural Sciences and Technology, Department of Plant Production and Technologies, Sivas, Turkey. E-mail: kahafe1974@yahoo.com DrcID: 0000-0001-5403-5629

<sup>&</sup>lt;sup>4</sup>Ridvan Ucar, Pamukkale University, Faculty of Agriculture, Department of Field Crops, Denizli, Turkey. E-mail: <u>rucar@pau.edu.tr</u> 🗓 OrcID: 0000-0001-6365-7200

<sup>&</sup>lt;sup>5</sup>Sam Mokhtarzadeh, Duzce University, Faculty of Agriculture, Department of Field Crops, Duzce, Turkey. E-mail: <u>sam.mokhtarzadeh@gmail.com</u> ២ OrcID: 0000-0002-3927-085

<sup>&</sup>lt;sup>6</sup>Muammer Ekmekci, Bingol University, Agricultural Research and Application Center Directorate, Bingol, Turkey. E-mail: <u>mekmekci@bingol.edu.tr</u> 🗓 OrcID:

<sup>0000-0002-0610-8552</sup> <sup>7</sup>Mehmet Ali Kutlu, Bingol University, Vocational School of Food, Agriculture and Livestock, Department of Crop and Animal Production, Bingol, Turkey. Email: malikutlu@bingol.edu.tr DrcID: 0000-0003-0862-9690

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# Öz

Bu çalışma; ak üçgülün farklı hasat zamanlarındaki verim ve kalite özelliklerinin belirlenmesi amacıyla yürütülmüstür. Çalışma, 2021 ve 2022 yıllarında tesadüf blokları deneme desenine göre yürütülmüş ve çalışmada bitkisel materyal olarak ak üçgülün Rivendel çeşidi kullanılmıştır. Araştırma konusu olarak, ak üçgülün erken çiçeklenme, yarı çiçeklenme, tam çiçeklenme ve çiçeklenme sonrası olmak üzere dört farklı hasat zamanı ele alınmıştır. Araştırmada ak üçgülün sahip olduğu bitki boyu, yeşil ot verimi, kuru ot verimi, kuru madde oranı, ham protein oranı, ham protein verimi, asit deterjanda çözünmeyen lif (ADF) oranı, nötr deterjanda çözünmeyen lif (NDF) oranı, nispi yem değeri ile fosfor, potasyum, kalsiyum ve magnezyum oranları incelenmiştir. İncelenen bu özelliklerin tümünün ak üçgülün farklı hasat dönemlerinde gösterdiği farklılığın istatistiksel olarak önemli olduğu belirlenmiştir. Araştırmada ak üçgül, her iki yılda da en yüksek bitki boyuna (32.3 cm ve 27.8 cm), yeşil ot verimine (38.367 kg ha<sup>-1</sup> ve 52.080 kg ha<sup>-1</sup>), kuru ot verimine (10.707 kg ha<sup>-1</sup> ve 13.424 kg ha<sup>-1</sup>) ve ham protein verimine (1987 kg ha<sup>-1</sup> ve 2544 kg ha<sup>-1</sup>) tam çiçeklenme aşamasında ulaşmıştır. 2021 yılında en yüksek ham protein ve nispi yem değeri ile en düşük ADF ve NDF oranları erken çiçeklenme ve yarı çiçeklenme, 2022 yılında ise bu özelliklere ait en yüksek değerler ise sadece erken çiçeklenme aşamalarından elde edilmiştir. Her iki yılda da en düşük fosfor ve potasyum oranları ile en yüksek kalsiyum ve magnezyum oranlarının çiçeklenme sonrası aşamadan elde edildiği belirlenmiştir. Sonuç olarak en yüksek verim değerleri ile ortalama kalite değerleri tam çiçeklenme aşamasından elde edildiğinden, ak üçgülün bu aşamada hasat edilmesinin üretici açısından daha avantajlı olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Hasat dönemi, Kuru ot, Ham protein, Nispi yem değeri, Makro elementler

#### 1. Introduction

Clover species, which are spread in the cool and humid regions of the temperate zone, produce very valuable and high-quality feed for animals because they have thin stems and abundant leaves (Onal Asci, 2016). White clover (*Trifolium repens* L.), which is among the clover species, is an important perennial legume forage plant with a wide distribution area, used for grazing, with very high nutritional value. White clover is highly resistant to grazing and chewing due to its horizontal development and stolon structure and has an indispensable place in forage crop production (Acikgoz, 2001; Acar and Ayan, 2012).

White clover (*Trifolium repens* L.) is a perennial forage legume plant commonly found in pastures, parks, and garden areas of our country (Demirkol and Yilmaz, 2018). At the same time, white clover is a species that can be used in the improvement of meadow and pastures (Röck Okuyucu and Okuyucu, 2006). Since the nutritive value of the white clover is high, the animals grazing in the pastures with the white and meadow clover can provide 40% more live weight gain than the animals grazing in the pastures where perennial grass is dominant (Kemp et al., 2010).

Forage crops can lose their nutritional value over time. In general, as the plant matures, there is a decrease in the protein content of the feed and the degree of digestion of the feed, while there is an increase in the NDF and ADF content. In other words, the quality of the feed decreases with the delay of the harvest time (Bayar and Cacan, 2019; Gursoy and Macit, 2020). Therefore, in order to obtain a quality roughage, it is necessary to determine the harvest time well and to know the quality characteristics of the plants in different developmental periods. In order to reveal the harvest time well, it is necessary to know the chemical composition of the forage are not likely to be the periods when they have the highest yield (Karayilanli and Ayhan, 2016). For this reason, it is necessary to know the period when forage crops are at the highest yield and highest quality and to harvest during this period.

The previous studies demonstrated that the effect of harvest time on the yield and quality of legume forage crops is important. In the study, in which the chemical structures of 11 *Astragalus* species were examined at different maturity stages, it was determined that the crude protein ratio decreased and the ratios of ADF and NDF increased from the pre-flowering to the fruit formation period (Cacan et al., 2017). In the study of determining the effect of harvest time on chemical composition of some leguminous plants, the chemical structure of white clover in vegetative, flowering and seed stages was investigated and it was determined that the crude protein ratio, which was 14.76% in the vegetative period, decreased to 12.13 % at the seed stage, the NDF ratio increased from 40.29% to 46.85%, and the ADF ratio increased from 32.45% to 44.01% (Ozkan et al., 2018). Bayar and Cacan (2019) determined that Hungarian vetch (*Vicia pannonica* Crantz.) harvested at different times had ideal values in terms of crude protein, ADF and NDF ratios during the flowering stage, and there was a decrease in grass yield and quality with the formation of pods in Hungarian vetch.

It was determined by previous studies that crude protein ratio, digestible dry matter ratio and relative feed value decreased, green forage and dry matter yield and ADF and NDF ratio increased in forage crops depending on the progress of the harvest time. However, the number of studies revealing the yield and quality characteristics of white clover in different harvest time is quite low. For this reason, the aim of this study is to reveal the yield and quality characteristics of white clover in different harvest time, and as a result, to determine the ideal harvest time.

#### 2. Materials and Methods

### 2.1. Research area and plant material

The research was conducted at Bingöl University Agricultural Application and Research Center between 2020-2022. This area is 15 km away from the city center of Bingöl (Türkiye), located at the coordinates of 38° 32' 41.85" N and 40° 32' 25.58" E and its height above sea level is 1080 m. Rivendel variety of white clover (*Trifolium repens* L.) was used as plant material in the research.

#### 2.2. Climatic characteristics of the research area

In the province of Bingöl, where the research was conducted, the annual average temperature value for many years is 12.3  $^{\circ}$ C. The average temperature was recorded as 14.5  $^{\circ}$ C in the years 2020-2021, when the research was conducted,

and 13.0 °C in the years 2021-2022. The average annual total precipitation of Bingöl province for many years is 932 mm. The most precipitation is received during the winter months. The average precipitation amount was recorded as 519 mm in the years 2020-2021, in which the research was conducted, and 960 mm in the years 2021-2022. It has been determined that the years 2020-2021 and 2021-2022 are warmer than the long-term average and the year 2020-2021 receives less precipitation than the long-term average (*Figure 1*).



Figure 1. Average temperature and precipitation values for the periods in which the research was conducted

#### 2.3. Soil characteristics of the research area

According to the results of the soil analysis made at the Faculty of Agriculture of Bingöl University; it has been seen that the research area was clay-loam, slightly acidic (pH: 6.26) and calcareous (0.41%), unsalted (0.014%), the amount of organic matter (1.09%) and potassium (18.27 kg da<sup>-1</sup>) was low and phosphorus (7.60 kg da<sup>-1</sup>) was medium.

### 2.4. Methods

The yield and quality characteristics of the hay cut at different harvest time of the white clover were evaluated in this study. For this purpose, the experiment was established on 02 June 2020 according to the randomized blocks design with four replications. In the experiment, 2.5 kg of seeds were used per decare (Marshall et al., 2004) and sowing was done by hand to a depth of 1-2 cm. With sowing, 4 kg of N and 10 kg of  $P_2O_5$  (9 kg da<sup>-1</sup> DAP) were given (Basbag et al., 2007). Row spacing was set at 40 cm and row length at 20 m in the field experiment. The field experiment was carried out in an area of 20 m x 20 m = 400 m<sup>2</sup>. The field experiment was conducted under irrigated conditions and no fertilization was applied except for the fertilization given with sowing. The year 2020 was accepted as the establishment year and no observation or result has been taken. On April 30, 2021, the first flowers began to appear in the trial area. In 2021 and 2022, white clover was harvested twice a year in early bloom, mid bloom, full bloom and after bloom stages. Harvesting was done on the dates and periods given in *Table 1* in 2021 and 2022.

| Table 1. Harvest dates | s of white cl | lover for 2021 | and 2022 |
|------------------------|---------------|----------------|----------|
|------------------------|---------------|----------------|----------|

|             | 2021 Harvest dates |            | 2022 Harvest dates |            |  |
|-------------|--------------------|------------|--------------------|------------|--|
|             | 1. Harvest         | 2. Harvest | 1. Harvest         | 2. Harvest |  |
| Early Bloom | 11.05.2021         | 09.08.2021 | 12.05.2022         | 01.08.2022 |  |
| Mid Bloom   | 01.06.2021         | 19.08.2021 | 30.05.2022         | 15.08.2022 |  |
| Full Bloom  | 25.06.2021         | 09.09.2021 | 16.06.2022         | 05.09.2022 |  |
| After Bloom | 08.07.2021         | 23.09.2021 | 30.06.2022         | 19.09.2022 |  |

The observations in the experiment were made on an area of  $1 \text{ m}^2$  determined in the parcels in four replications. Plant height was measured in cm, with 10 plants per replication (Basbag et al., 2007).  $1 \text{ m}^2$  area was harvested and the green forage obtained from this area was weighed and green forage yield was obtained. 500 g samples were taken from the green forage obtained from this area and dried at 70 °C for 48 hours. Dry matter yield was calculated by multiplying the results obtained with the green forage yield (Cacan et al., 2018).

Hay samples belonging to the plots whose dry matter content was determined were grinded separately for each plot and made ready for analysis. Crude protein (CP), insoluble fiber in acid detergent (ADF), insoluble fiber in neutral detergent (NDF), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) ratios of ground samples by Near Infrared Reflectance Spectroscopy (NIRS) instrument with #IC-0904FE calibration have determined (Brogna et al., 2009). Relative feed value determined with the help of the formula (Eq. 1);

 $RFV = ((120/NDF) \times (88.9 - (0.779 \times ADF)) \div 1.29)) \text{ (Van Dyke and Anderson, 2000)}$ (Eq. 1)

Two harvests were made in both years of the study. The research data were obtained by taking the average of two harvests in each year. Analysis of variance was applied to the obtained data according to the randomized blocks experimental design with JPM statistical program. The differences of the means were compared with the LSD test at the 0.05 level (Acikgoz and Acikgoz, 2001).

### 3. Results and Discussion

White clover planting was done on 02 June 2020. Since the first year is the establishment year, a harvest was made and no observations were taken. In 2021 and 2022, two harvests were taken in the ecological conditions of the region. In a previous study, it was reported that two harvests of white clover were obtained after the first year in Adana and Hatay provinces (Tukel et al., 2001). Yield and quality characteristics and macro element contents obtained from white clover in 2021, 2022 and two-year average were given in *Table 2*. The green forage and dry matter yields given in *Table 2* were given as the sum of the two cutting made during the year, and the other characteristics were given as the average of the two cutting made during the year.

## 3.1. Yield characteristics

Yield characteristics obtained from white clover in 2021, 2022 and two-year average were given in *Table 2* and *Figure 2*. It was observed that the difference in plant height, green forage and dry matter yields from the yield characteristics examined in different development periods of white clover in 2021, 2022 and two-year average were statistically significant. Additionally, the differences between years for yield characteristics, and the interaction between year x bloom in terms of green forage and dry matter yields were statistically significant ( $P \le 0.01$ ).

The lowest values of plant height in white clover were taken from early and mid-bloom periods in 2021, 2022 and two-year average, and the highest plant height was taken from full bloom and after bloom periods. The average plant height of white clover was 27.6 cm in 2021, 23.3 cm in 2022 and 25.5 cm in two-year average, as the average of all developmental stages. From the perspective of two years, it was seen that the white clover elongates a little in the early and mid-bloom periods and reaches the highest plant height in the full bloom period. At the after-bloom stage, it was observed that the plant height decreased due to the plant's water loss, but this decrease was not statistically significant (*Table 2, Figure 2*).

Plant height of white clover in Türkiye was 26.32 cm in average in the ecological conditions of the Eastern Anatolia Region (Aygun and Olgun, 2014), 25-40 cm in ecological conditions of the Black Sea Region (Samsun province) (Basaran et al., 2006), 9.88-14.56 cm in the ecological conditions of the Southeastern Anatolia Region (Basbag et al., 2007) and 18.0-31.0 cm in the ecological conditions of the Mediterranean Region (Antalya province) (Demiroglu and Avcioglu, 2010; Oten et al., 2019). It was seen that the plant height obtained from the white clover was similar to the average plant height obtained from the Eastern Anatolia, Black Sea, and Mediterranean Regions, but higher than the average plant height obtained from the Southeastern Anatolia Region. The probable reason for this height is that the Eastern Anatolia Region receives more precipitation than the Southeastern Anatolia Region. The fact that the region receives more precipitation has led to higher plant height, which is one of the yield elements.

It was seen that the green forage and dry matter yields start to increase after the early bloom period in 2021, 2022 and two-year average, reach the highest level in the full bloom period, and both the green forage yield and the dry matter yield decrease rapidly in the after-bloom stage. It was seen that the difference between full bloom and after bloom stage of green forage yield obtained only in 2021 was statistically in the same group. As the average of the development stages of white clover, the green forage yield was 26.008 kg ha<sup>-1</sup> in 2021, 38.057 kg

Cacan & Ozdemir & Kokten & Ucar & Moktarzadeh & Ekmekci & Kutlu Change of Forage Yield and Quality Characteristics of White Clover (*Trifolium repens* L.) at Different Harvest Time ha<sup>-1</sup> in 2022 and 32.032 kg ha<sup>-1</sup> in two-year average. Additionally, the dry matter yield was 6.585 kg ha<sup>-1</sup> in 2021, 9.296 kg ha<sup>-1</sup> in 2022 and 7.940 kg ha<sup>-1</sup> in two-year average (*Table 2, Figure 2*).

|         | Veer 2021                                | Early      | Mid      | Full     | After    | A       |  |
|---------|--|------------|----------|----------|----------|---------|--|
|         | Year 2021                                | Bloom      | Bloom    | Bloom    | Bloom    | Average |  |
| /ield   | Plant height, cm                         | 23.4 b**   | 23.5 b   | 32.3 a   | 31.1 a   | 27.6    |  |
|         | Green forage yield, kg ha <sup>-1</sup>  | 13.013 b** | 15.960 b | 38.367 a | 36.693 a | 26.008  |  |
| Y       | Dry matter yield, kg ha <sup>-1</sup>    | 3.064 b**  | 4.622 b  | 10.707 a | 7.946 a  | 6.585   |  |
|         | Dry matter, %                            | 89.5 c**   | 89.8 b   | 89.8 b   | 90.1 a   | 89.8    |  |
| Quality | Crude protein, %                         | 21.4 a*    | 20.0 ab  | 18.6 bc  | 17.4 c   | 19.4    |  |
|         | Crude protein yield, kg ha <sup>-1</sup> | 657 c**    | 924 bc   | 1987 a   | 1385 b   | 1238    |  |
|         | ADF, %                                   | 21.3 c**   | 21.0 c   | 26.5 b   | 28.2 a   | 24.3    |  |
|         | NDF, %                                   | 29.7 b**   | 28.8 b   | 34.5 a   | 35.8 a   | 32.2    |  |
|         | RFV                                      | 227 a**    | 235 a    | 184 b    | 174 b    | 205     |  |
| Ŭ       | Phosphorus, %                            | 0.34 a*    | 0.31 ab  | 0.31 ab  | 0.29 b   | 0.31    |  |
|         | Potassium, %                             | 2.25 a**   | 2.12 a   | 2.07 a   | 1.59 b   | 2.01    |  |
|         | Calcium, %                               | 1.81 c**   | 1.92 b   | 1.92 b   | 2.05 a   | 1.93    |  |
|         | Magnesium, %                             | 0.39 c**   | 0.40 c   | 0.42 b   | 0.47 a   | 0.42    |  |
|         | Very 2022                                | Early      | Mid      | Full     | After    | A       |  |
|         | 1 ear 2022                               | Bloom      | Bloom    | Bloom    | Bloom    | Average |  |
| q       | Plant height, cm                         | 19.3 b**   | 20.1 b   | 27.8 a   | 26.0 a   | 23.3    |  |
| Yield   | Green forage yield, kg ha <sup>-1</sup>  | 35.880 c** | 40.293 b | 52.080 a | 23.973 d | 38.057  |  |
|         | Dry matter yield, kg ha <sup>-1</sup>    | 7.143 c**  | 9.743 b  | 13.424 a | 6.873 c  | 9.296   |  |
|         | Dry matter, %                            | 89.0 c**   | 89.3 b   | 89.3 b   | 89.5 a   | 89.3    |  |
|         | Crude protein, %                         | 23.8 a**   | 21.8 b   | 19.0 c   | 18.3 c   | 20.7    |  |
|         | Crude protein yield, kg ha <sup>-1</sup> | 1702 c**   | 2121 b   | 2544 a   | 1259 d   | 1906    |  |
|         | ADF, %                                   | 17.6 c**   | 20.9 b   | 23.2 a   | 24.6 a   | 21.6    |  |
| llity   | NDF, %                                   | 26.5 c**   | 30.0 b   | 31.5 a   | 32.1 a   | 30.0    |  |
| Qua]    | RFV                                      | 264 a**    | 226 b    | 209 c    | 202 c    | 225     |  |
| Ŭ       | Phosphorus, %                            | 0.36 a**   | 0.32 b   | 0.30 c   | 0.30 c   | 0.32    |  |
|         | Potassium, %                             | 2.39 a**   | 2.22 ab  | 2.05 b   | 1.68 c   | 2.09    |  |
|         | Calcium, %                               | 1.85 c**   | 1.93 b   | 1.91 bc  | 2.06 a   | 1.94    |  |
|         | Magnesium, %                             | 0.38 b**   | 0.38 b   | 0.40 b   | 0.45 a   | 0.40    |  |
|         | Two-Vear Average                         | Early      | Mid      | Full     | After    | Average |  |
|         | Two-Tear Average                         | Bloom      | Bloom    | Bloom    | Bloom    |         |  |
| q       | Plant height, cm                         | 21.4 c**   | 21.8 c   | 30.1 a   | 28.6 b   | 25.5    |  |
| í iel   | Green forage yield, kg ha <sup>-1</sup>  | 24.447 b** | 28.127 b | 45.223 a | 30.333 b | 32.032  |  |
|         | Dry matter yield, kg ha <sup>-1</sup>    | 5.104 c**  | 7.182 b  | 12.065 a | 7.409 b  | 7.940   |  |
|         | Dry matter, %                            | 89.3 c**   | 89.6 b   | 89.6 b   | 89.8 a   | 89.5    |  |
|         | Crude protein, %                         | 22.6 a**   | 20.9 b   | 18.8 c   | 17.9 c   | 20.0    |  |
|         | Crude protein yield, kg ha <sup>-1</sup> | 1180 b**   | 1522 b   | 2265 a   | 1322 b   | 1572    |  |
| >       | ADF, %                                   | 19.4 c**   | 20.9 c   | 24.9 b   | 26.4 a   | 22.9    |  |
| ality   | NDF, %                                   | 28.1 b**   | 29.4 b   | 33.0 a   | 33.9 a   | 31.1    |  |
| Quí     | RFV                                      | 245 a**    | 230 b    | 197 c    | 188 c    | 215     |  |
|         | Phosphorus, %                            | 0.35 a**   | 0.32 b   | 0.30 bc  | 0.30 c   | 0.32    |  |
|         | Potassium, %                             | 2.32 a**   | 2.17 ab  | 2.06 b   | 1.64 c   | 2.05    |  |
|         | Calcium, %                               | 1.83 c**   | 1.92 b   | 1.91 b   | 2.05 a   | 1.93    |  |
|         | Magnesium, %                             | 0.39 c**   | 0.39 c   | 0.41 b   | 0.46 a   | 0.41    |  |

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| <i>adie 2</i> . | rieia ana | quanty | characteristics      | oj wnue               | clover a | <i>i aijjereni</i> | narvesi ui | ne |

\*: P≤0.05, \*\*: P≤0.01, LSD (Years): \*\*, LSD (Bloom): \*\*, LSD (Years x Bloom): \*\*

Year x bloom interaction was found significant only for green forage and dry matter yields. In terms of interactions, the highest green forage and dry matter yields were obtained from the full bloom period of 2022, while the lowest values were obtained from the early bloom period of 2021.

The green forage yield (26.008 kg ha<sup>-1</sup>) obtained in 2021 increased by 46% to 38.057 kg ha<sup>-1</sup> in 2022, and the dry matter yield (6.585 kg ha<sup>-1</sup>) obtained in 2021 increased by approximately 41% in 2022 to 9.296 kg ha<sup>-1</sup> (*Figure 2*). Perennial legume forage crops show poor growth in the first year. Therefore, the first year is considered the plant year and is not evaluated in terms of yield or quality. Perennial legume forage crops start to show their main yield after the second year and give higher yields in the following years (Acar and Onal Asci, 2006). There is a similar situation in this study. This year of the white clover, which was sowing in 2020, was accepted as the year of establishment and no observations were taken. In 2021, the first observations began to be taken. Due to its nature, higher values were obtained in 2022 from white clover, which is a legume forage plant.



Figure 2. Changes in plant height, green forage yield, dry matter yield and dry matter ratios of white clover in 2021 and 2022

When the studies on green forage and dry matter yields in white clover were examined; under Mediterranean and Southeastern Anatolia Region conditions (Adana, Hatay and Şanlıurfa), green forage yield 993-4159 kg ha<sup>-1</sup>, dry matter yield 150-696 kg ha<sup>-1</sup> (Tukel et al., 2001), in Black Sea Region (Samsun province) ecological conditions green forage yield 1784 kg ha<sup>-1</sup> and dry matter yield 432 kg ha<sup>-1</sup> (Acar and Onal Asci, 2006), in Southeastern Anatolia Region conditions (Diyarbakır province) green forage yield 17713-28490 kg ha<sup>-1</sup> and dry matter yield 4501-7574 kg ha<sup>-1</sup> (Basbag et al., 2007), in Mediterranean Region climate conditions green forage yield 1061-1735 kg ha<sup>-1</sup> and dry matter yield 204-343 kg ha<sup>-1</sup> were reported (Demiroglu and Avcioglu, 2010). In a similar study, it was reported that in the experiment conducted in the Black Sea region, two harvests were taken and the dry matter yield of white clover was 549 kg ha<sup>-1</sup> in the first year and 463 kg ha<sup>-1</sup> in the second year (Can and Ayan, 2020). In a three-year study conducted in England (Marshall et al., 2003), the dry matter yield of *Trifolum repens* was determined as 4556-5928 kg ha<sup>-1</sup>. It was seen that the green forage and dry matter yields obtained from this study showed partial similarities with the previous studies.

### 3.2. Quality characteristics

The quality characteristics (dry matter, crude protein, crude protein yield, ADF, NDF and relative feed value) of white clover determined in 2021, 2022 and two-year average were given in Table 2 and Figure 3. It was seen that the difference between dry matter, crude protein, crude protein yield, NDF, ADF ratios and relative feed value from the properties of white clover examined in different development periods is statistically significant in 2021, 2022 and two-year average. Additionally, the differences between years for quality characteristics, and the interaction between year x bloom in terms of crude protein yield, NDF, ADF and relative feed value were statistically significant ( $P \le 0.01$ ).



Figure 3. Changes in NDF, ADF and CP ratios and relative feed value of white clover in 2021 and 2022

Dry matter is dehydrated feed. It is obtained by subtracting the moisture content from 100 and its average value in feeds varies between 85-92% (Rivera and Parish, 2010). It was seen that the dry matter ratio increased continuously from early bloom to after bloom in both years and reached its highest values after bloom. As the average of the development stages of white clover, the dry matter ratio was 89.8% in 2021, 89.3% in 2022 and 89.5% in two-year average. It was seen that the dry matter ratio increased after early bloom in white clover, did not change in half and full bloom periods, and increased again after bloom. The dry matter ratio is related to the water loss of the white clover. It was found out that as the plant matures, the amount of water it contains decreases (*Table 2, Figure 2*). Similar to the study findings Demirel et al. (2010) reported that dry matter rate of white clover is 92.58%. Kurt et al. (2022) observed that dry matter ratio in white clover is 91.8%. Also, Ozkan et al. (2018), reported that the dry matter ratio increased from the vegetative period (92.96%) to the seed stage (94.01%) in white clover.

In 2021, 2022 and two-year average, it was observed that the white clover has the highest crude protein ratio in the early bloom period, the crude protein ratio decreases in the mid-bloom stage and has the lowest crude protein ratio in the full bloom and after bloom. As the average of the developmental stages of white clover, the crude protein ratio was 19.4% in 2021, 20.7% in 2022 and 20.0% in two-year average. It is understood that as the plant matures, the crude protein content decreases regularly. The highest crude protein yield was obtained from the full bloom stage in both years and two-year average. The average crude protein yield from white clover was 1238 kg

ha<sup>-1</sup> in 2021, 1906 kg ha<sup>-1</sup> in 2022 and 1572 kg ha<sup>-1</sup> in two-year average (*Table 2, Figure 3*). Year x bloom interaction was found significant for crude protein yield. The highest crude protein yield was obtained from the full bloom period of 2022, while the lowest value was obtained from the early bloom period of 2021.

Similar to the findings of the study, the crude protein ratio in white clover; Marshall et al. (2004) 16.9-23.6% in England, Acar and Onal Asci (2006) %20.38 in the Black Sea region, Basaran et al. (2006) %18.93, Basbag et al. (2007) %16.52-19.00 and Basbag et al. (2011) %19.41 in the conditions of the Southeastern Anatolia Region, Ergon et al. (2016) %16.3-22.6 in Norway and Kurt et al. (2022) %17.9 in the conditions of the Eastern Anatolia Region have been detected. These findings obtained by the researchers are similar to the findings of the current study. Also, in a previous study, it was determined that the crude protein ratio in white clover decreased regularly from the vegetative period to the seed stage (Ozkan et al., 2018).

The lowest NDF and ADF contents in white clover were taken from early bloom and mid bloom in both years and two-year average, and the highest NDF and ADF contents were from full bloom and after-bloom stages. In general, it was found out that NDF and ADF contents increase as the plant matures. As the average of the developmental stages of white clover, the NDF content was 32.2% in 2021, 30.0% in 2022, %31.1 in two-year average and the ADF content was 24.3% in 2021, 21.6% in 2022 and 22.9% in two-year average (*Table 2, Figure 3*). Year x bloom interaction was found significant for NDF and ADF contents. The highest NDF and ADF contents were obtained from the full and after bloom period of 2021, while the lowest values were obtained from the early bloom period of 2021.

In the white clover; in Southeastern Anatolia Region conditions, ADF rate 24.71%, NDF rate 35.0% (Basbag et al., 2011), in the conditions of the Eastern Anatolia Region, the ADF rate 28.08% and the NDF rate 43.62% (Cacan et al., 2015), in a study conducted in Norway, NDF rate 36.9-43.8%, ADF rate 26.9-29.3% (Ergon et al., 2016), in Black Sea conditions, ADF content 23.8%-26.4%, NDF content 33.2%-37.6% (Can and Ayan, 2020), in the conditions of the Eastern Anatolia Region, the ADF rate 24.7% and the NDF rate 36.7% (Kurt et al., 2022) was determined. It was observed that the NDF and ADF ratios obtained in white clover are partially similar to the findings of other studies.

Relative feed value is a parameter that describes the response of animals to feed quality. Relative feed value provides an estimate of the expected feed intake and energy value from a feed. Relative feed value is calculated based on the ADF and NDF ratio of alfalfa in full bloom and a value above 151 is considered "prime" (Rivera and Parish, 2010). The highest relative forage value in white clover was obtained from early bloom and mid-bloom stages in 2021, and only from early bloom stage in 2022 and two-year average. It was observed that higher relative feed value was obtained from the early stages of white clover, the relative feed value decreased as the plant matured, and the lowest values were obtained after bloom. It was determined that the relative feed values obtained even in the after-bloom stage, where the lowest values were obtained, were above 151 and included in the "prime" group. As the average of the development stages of the white clover, the relative feed value was obtained as 205 in 2021, 225 in 2022 and 2015 two-year average (*Table 2, Figure 3*). Year x bloom interaction was found significant for relative feed value. The highest relative feed value was obtained from the after bloom period of 2022, while the lowest value was obtained from the after bloom period of 2022.

Can and Ayan (2020) determined the relative feed value in white clover in the range of 153-211. Kurt et al. (2022), reported that white clover has a higher value in terms of digestibility and relative feed value than other leguminous forage plants, and that they have determined the relative feed value as 176. These values obtained from the white clover were found close to the findings obtained from the study. The relative feed value obtained as 142 by Bozkurt Kiraz (2011) and as 143 by Cacan et al. (2015) was found to be lower than the finding obtained from the experiment.

The macro element contents obtained from white clover in 2021, 2022 and two-year average were given in *Table 2* and *Figure 4*. It is seen that the difference between phosphorus, potassium, calcium and magnesium contents of macro elements examined at different developmental levels of white clover was statistically significant in 2021, 2022 and two-year average. It was observed that only the difference in Mg content was statistically significant in terms of years. Year x bloom interaction was found insignificant for all macro elements.

#### Cacan & Ozdemir & Kokten & Ucar & Moktarzadeh & Ekmekci & Kutlu Change of Forage Yield and Quality Characteristics of White Clover (*Trifolium repens* L.) at Different Harvest Time

In white clover, the highest phosphorus and potassium contents and the lowest calcium and magnesium contents were obtained from the early, mid and full bloom stages, and the highest calcium and magnesium contents and the lowest phosphorus and potassium contents were obtained from the after-bloom stage in 2021. In 2021, the average of the developmental stages of the white clover was phosphorus 0.31%, the potassium was 2.01%, the calcium was 1.93%, and the magnesium was 0.42% (Table 2). In white clover, in 2022, the highest phosphorus ratio was obtained from early bloom, the highest potassium was obtained from early and mid-bloom, and the highest calcium and magnesium contents were obtained from the after-bloom stages. The lowest phosphorus was obtained from full and after bloom, the lowest potassium was obtained after bloom, the lowest calcium was obtained from early bloom and the lowest magnesium was obtained from early, mid, and full bloom stages. In 2022, the average of the developmental stages of white clover was phosphorus of 0.32%, potassium of 2.09%, calcium of 1.94% and magnesium of 0.40%. In the two-year average, the highest phosphorus and potassium contents were obtained from early bloom stages and highest calcium and magnesium contents were obtained from the after bloom stages. In the two-year average, the average of the developmental stages of white clover was phosphorus of 0.32%, potassium of 2.05%, calcium of 1.93% and magnesium of 0.41% (Table 2). In both years, it was observed that the phosphorus and potassium contents of white clover tend to decrease as the plant matures, while the calcium and magnesium contents tend to increase with the maturation of the plant (Figure 4).



Figure 4. Changes in P, K, Ca and Mg content of white clover in 2021 and 2022

It was observed that the research findings were similar to the contents of phosphorus (0.41%), potassium (2.70%), calcium (1.48%) and magnesium (0.31%) obtained from white clover in the Southeastern Anatolia Region conditions (Basbag et al., 2011), the ratios of phosphorus (0.63%), calcium (1.34%) and magnesium (0.29%) obtained from white clover in the conditions of the Eastern Anatolia Region (Cacan et al., 2015), the ratios of phosphorus (0.38-0.41%), potassium (2.44-2.86%), calcium (1.57-1.58%) and magnesium (0.29-0.30%) obtained from alfalfa in the conditions of the Middle Anatolia Region (Engin and Mut, 2018) and the ratios of

phosphorus (0.27%), potassium (1.49%), calcium (1.63%) and magnesium (0.36%) obtained from Astragalus taxa in the conditions of Eastern Anatolia Region (Çaçan et al., 2023).

The contents of P, K, Ca and Mg obtained from white clover were found to be in line with the optimal ratios of 0.2-0.5% for phosphorus, 1.0-5.0% for potassium and 0.1-0.4% for magnesium, reported by Motsara and Roy (2008). However, the calcium contents obtained from white clover in 2021, 2022 and two-year average were found to be higher than the 0.1-1.0% calcium content reported as the optimal ratio reported by Motsara and Roy (2008). In terms of the health of animals, there must be a balance between the minerals in the feed. In this study, the high Ca ratio causes an imbalance between Ca:P. In general, a 2:1 ratio of Ca:P in feeds is ideal and its excess causes milk fever in animals (Acikgoz, 2001). However, if the animals receive enough vitamin D, this ratio can be tolerated up to 7:1 (Barnes et al., 1990; Buxton and Fales, 1994). In this respect, the Ca:P ratio obtained was found to be tolerable.

### 4. Conclusions

This study investigated the effect of different harvest time of white clover on yield and quality, as the average of two years, it was observed that the highest values in terms of yield were taken from the full bloom stage. The highest crude protein, relative feed value, phosphorus and potassium ratios and the lowest dry matter, NDF and ADF contents were obtained from the early bloom period. Although the early bloom stage gave higher values in terms of these characteristics, it is clear that up to 70% yield loss will occur in the harvest made at this stage. However, it was found out that even in a harvest to be made at the full bloom stage, white clover will be in the "prime" group in terms of relative feed value and the highest values will be obtained in terms of yield. For this reason, it was concluded that it would be more advantageous to harvest at the stage of full bloom, which has the highest yield and can be considered ideal in terms of quality.

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# **Ethical Statement**

There is no need to obtain permission from the ethics committee for this study.

# **Conflicts of Interest**

We declare that there is no conflict of interest between us as the article authors.

# Authorship Contribution Statement

Concept: Cacan, E., Ozdemir, S., Kokten, K., Ucar, R., Mokhtarzadeh, S., Ekmekci, M., Kutlu, M. A.; Design: Cacan, E., Ozdemir, S., Kokten, K., Ucar, R., Mokhtarzadeh, S., Ekmekci, M., Kutlu, M. A.; Data Collection or Processing: Ozdemir, S., Ucar, R., Ekmekci, M.; Statistical Analyses: Cacan, E., Kokten, K.; Literature Search: Mokhtarzadeh, S., Kutlu, M. A.; Writing, Review and Editing: Cacan, E., Ozdemir, S., Kokten, K., Ucar, R., Mokhtarzadeh, S., Ekmekci, M., Kutlu, M. A.; Mokhtarzadeh, S., Ekmekci, M., Kutlu, M. A.

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