



## Comparison of chemical composition and nutritive values of some clover species

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## ABSTRACT

This study was carried out to compare the chemical composition and nutritive value of some clover species, especially common in the rangelands of the Mediterranean region. For this purpose, clover species (*Trifolium angustifolium*, *Trifolium cherleri*, *Trifolium hybridum*, *Trifolium lappaceum*, *Trifolium nigrescens*, *Trifolium pilulare*, *Trifolium resupinatum*, *Trifolium scabrum*, *Trifolium spumosum* and *Trifolium tomentosum*) were collected from ten different points in Hatay Mustafa Kemal University campus pasture. To determine the chemical composition of collected clover species, crude ash (CA), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), hemicellulose (Hcel) and cellulose (Cel) contents were investigated. In addition, dry matter digestibility (DMD), dry matter intake (DMI), relative feed value (RFV) and net energy lactation (NEL) characteristics were calculated in order to determine the nutritional value of clovers. Differences among species in terms of all features examined were found to be statistically significant. CA, CP, NDF, ADF, ADL, Hcel and Cel contents of species varied between 5.36% and 9.85%, 18.47% and 22.05%, 30.31% and 49.80%, 21.32% and 34.28, 3.25% and 5.04, 8.99% and 15.97% and 18.07% and 30.38, respectively. It was determined that the values of DMD, DMI, RFV and NEL properties calculated for the nutritive values of these plants varied between 62.20-72.29%, 2.41-3.97%, 116.20-222.18 and 1.40-1.74%, respectively. When the results obtained from the study were evaluated collectively, it was determined that *T. resupinatum* and *T. hybridum* offered a superior nutrient content and nutritional value compared to other species.

**Keywords:** Chemical Composition, Nutritional Value, Clover Species, Rangeland, Forage.

## Bazı üçgül türlerinin kimyasal kompozisyonu ve besin değerlerinin karşılaştırılması

## ÖZ

Bu çalışma özellikle Akdeniz iklim bölgesi meralarında yaygın bir şekilde bulunan bazı üçgül türlerinin kimyasal kompozisyonunu ve besin değerini karşılaştırmak için yürütülmüştür. Bu amaçla Hatay Mustafa Kemal Üniversitesi kampüs merasında on farklı noktadan üçgül (*Trifolium angustifolium*, *Trifolium cherleri*, *Trifolium hybridum*, *Trifolium lappaceum*, *Trifolium nigrescens*, *Trifolium pilulare*, *Trifolium resupinatum*, *Trifolium scabrum*, *Trifolium spumosum* ve *Trifolium tomentosum*) türleri toplanmıştır. Toplanan bu üçgül türlerinde kimyasal kompozisyonu belirlemek için ham kül (HK), ham protein (HP), nötr ortamda çözünmeyen lif (NDF), asitli ortamda çözünmeyen lif (ADF), asitli ortamda çözünmeyen lignin (ADL), hemiselüloz (Hsel) ve selüloz (Sel) içerikleri incelenmiştir. Ayrıca besin değerini belirlemek için kuru madde sindirimi (KMS), kuru madde tüketimi (KMT), nispi yem değeri (NYD) ve net enerji laktasyon (NEL) özellikleri hesaplanmıştır. Tek yıllık üçgül türlerinde incelenen tüm bu özellikler istatistiki açıdan önemli bulunmuştur. Türlerin HK, HP, NDF, ADF, ADL, Hsel ve Sel içerikleri sırasıyla %5.36-9.85, %18.47-22.05, %30.31-49.80, %21.32-34.28, %3.25-5.04, %8.99-15.97 ve %18.07-30.38 arasında değişiklik göstermiştir. Bu bitkilerin besin değerleri için hesaplanan KMS, KMT, NYD ve NEL özelliklerine ait değerlerin sırasıyla %62.20-72.29, %2.41-3.97, 116.20-222.18 ve %1.40-1.74 arasında değiştiği belirlenmiştir. Çalışmadan elde edilen bulgular topluca değerlendirildiğinde *T. resupinatum* ile *T. hybridum* türlerinin diğer türlere kıyasla üstün bir besin madde içeriği ve besin değerlerine sahip olduğu belirlenmiştir.

**Anahtar Kelimeler:** Kimyasal Kompozisyon, Besin Değeri, Tek Yıllık Üçgül Türleri, Mera, Yem.

## 1. INTRODUCTION

In general, legumes are widely used as green fodder, silage, ornamental plant, soil improvement, pollen and

nectar source and grazing plant in pasture vegetation.<sup>1</sup> Legume forage crops offer significant advantages in replanting winter pastures in arid regions.<sup>2</sup> In addition, nitrogen fixation to the soil where legumes grown can

provide nitrogen to many grasses.<sup>3</sup> Among the perennial forage crops, perennial legumes are considered the main roughage source for livestock and are cultivated worldwide.<sup>1</sup> Clover species are accepted as very valuable plants especially in many countries where Mediterranean climate prevails.<sup>2</sup> These plants are especially common in the pastures of our Mediterranean climate regions in which the pastures stated that the plant material collected from.

One of the most important criteria affecting forage quality in legume forage crops is species diversity. Species-dependent variation is primarily due to the morphology of shoot growth.<sup>4</sup> The difference in forage quality between species may also be caused by the difference in leaf and stem ratios of plant species.<sup>5</sup> Knowing the feed quality and acting with this awareness can increase the final animal productivity.<sup>6</sup>

The most commonly used analysis methods for the determination of feed quality can be listed as raw ash, which gives an idea about the mineral content of the feed, nitrogen determination that shows the protein content, insoluble fiber in neutral medium, insoluble fiber in acidic medium, and insoluble lignin in acidic medium.<sup>6-8</sup> Based on these analyzes, the nutritional value of the feeds is calculated and presented as various characteristics theoretically.<sup>9</sup> Thus, it is possible to have an idea about the nutrient content and feed quality of the roughage obtained from forage plants.

In this study, the chemical composition and nutritive value of some clover species, which are especially common in the rangelands of the Mediterranean climate regions, were investigated and the plant species were compared in terms of these characteristics.

## 1. MATERIALS AND METHODS

In this study, some clover species (*Trifolium angustifolium*, *Trifolium cherleri*, *Trifolium hybridum*, *Trifolium lappaceum*, *Trifolium nigrescens*, *Trifolium pilulare*, *Trifolium resupinatum*, *Trifolium scabrum*, *Trifolium spumosum* and *Trifolium tomentosum*) were used as plant material. These plant species were collected from Hatay Mustafa Kemal University campus pasture from 30 March to 15 April from 10 different points during the flowering period by cutting from a height of 5 cm from the soil surface. The same plant species collected from the different points were mixed and left to

dry in a hot air forced oven at 65 °C for 48 hours with three repetitions. The dried plant samples were theoretically ground at a diameter of 1 mm in the mill to make them ready for chemical analysis. Dry matter was determined in the dried samples in ground air. For the determination of dry matter (DM), 1 g of ground plant samples were weighed and dried in porcelain crucibles in a hot air blowing oven at 105 °C for at least 4 hours,<sup>10</sup> and dry matter determination was calculated. All feed quality and nutritional value criteria given in % symbol were calculated on the basis of dry matter. Crude protein (CP) and crude ash (CA) contents of clover species were determined according to the method of AOAC.<sup>10</sup> The Kjeldahl method was applied to determine the nitrogen content and it was multiplied by 6.25 to calculate the crude protein content.<sup>10</sup> The ground plant samples, which were weighed into porcelain crucibles as 1 g for CA analysis, were burned in a muffle furnace at 550 °C. Based on the analysis of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL), cell wall components of clover species were determined according to the method of Van Soest et al.<sup>11</sup> In addition, hemicellulose (Hcel) and cellulose (Cel) were calculated using the difference between NDF and ADF and the difference between ADF and ADL in these plant species.<sup>11</sup> According to Van Dyke and Anderson,<sup>9</sup> the dry matter digestibility (DMD), dry matter intake (DMI), relative feed value (RFV) and net energy lactation (NEL) characteristics of clover species were calculated with the following formulas.

$$\text{DMD} = 88.9 - (0.779 \times \% \text{ADF})$$

$$\text{DMI} = 120 / \% \text{NDF}$$

$$\text{RFV} = \% \text{DMD} \times \% \text{DMI} \times 0.775$$

$$\text{NEL} = (1.044 - (0.0119 \times \% \text{ADF})) \times 2.205$$

ANOVA test was applied to all numerical data obtained from this study and the features found to be significant ( $p \leq 0.05$ ) as a result of this test were grouped with Tukey pairwise test.

## 2. RESULTS AND DISCUSSION

The F values and significance levels of the chemical composition and nutritional value properties of some clover species were given in Table 1. As seen from Table 1, among the clover species, all the properties examined in terms of chemical composition and nutritional value were found to be statistically significant.

**Table 1.** ANOVA test results of chemical composition and nutritional value properties of some clover species.

	DM	CA	CP	NDF	ADF	ADL	Hcel	Cel	DMD	DMI	RFV	NEL
F	14.02	548.21	72.33	68.03	316.13	30.23	10.81	309.52	319.00	53.44	76.73	288.26
P	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
CV	0.21	1.00	1.08	2.63	1.35	4.41	8.15	1.54	0.45	2.59	2.80	0.69

Data on DM, CA, CP, NDF, ADF and ADL characteristics of some clover species and mean comparison test results were given in Table 2. The DM contents of the clover species evaluated in this study

varied between 94.84% and 96.74%. The highest DM was detected in *T. angustifolium* and the lowest in *T. resupinatum*. It has been reported in many studies that DM contents vary depending on plant species.<sup>12-14</sup>

**Table 2.** Mean comparison test results of DM, CA, CP, NDF, ADF and ADL traits in some clover species.

Clover species	DM %	CA %	CP %	NDF %	ADF %	ADL %
<i>T. angustifolium</i>	96.74±0.29 <sup>a</sup>	7.65±0.02 <sup>e</sup>	19.30±0.08 <sup>e</sup>	43.71±0.08 <sup>e</sup>	30.71±0.23 <sup>c</sup>	4.54±0.02 <sup>bc</sup>
<i>T. cherleri</i>	95.70±0.08 <sup>b</sup>	7.58±0.07 <sup>e</sup>	19.41±0.17 <sup>e</sup>	49.80±0.71 <sup>a</sup>	34.28±0.33 <sup>a</sup>	3.90±0.05 <sup>cde</sup>
<i>T. hybridum</i>	95.85±0.08 <sup>b</sup>	9.85±0.05 <sup>a</sup>	21.93±0.09 <sup>ab</sup>	38.60±1.59 <sup>d</sup>	28.07±0.35 <sup>d</sup>	5.43±0.26 <sup>a</sup>
<i>T. lappaceum</i>	95.87±0.05 <sup>b</sup>	5.36±0.07 <sup>f</sup>	18.47±0.14 <sup>f</sup>	48.56±0.10 <sup>ab</sup>	32.59±0.32 <sup>b</sup>	3.63±0.21 <sup>de</sup>
<i>T. nigrescens</i>	96.19±0.13 <sup>ab</sup>	8.94±0.04 <sup>c</sup>	20.90±0.09 <sup>cd</sup>	37.29±0.38 <sup>de</sup>	26.20±0.13 <sup>e</sup>	4.39±0.08 <sup>bc</sup>
<i>T. pilulare</i>	95.67±0.16 <sup>b</sup>	7.47±0.01 <sup>e</sup>	19.37±0.10 <sup>e</sup>	45.37±0.49 <sup>bc</sup>	31.68±0.20 <sup>bc</sup>	5.04±0.07 <sup>ab</sup>
<i>T. resupinatum</i>	94.84±0.09 <sup>c</sup>	8.41±0.11 <sup>d</sup>	22.05±0.26 <sup>a</sup>	30.31±0.85 <sup>f</sup>	21.32±0.17 <sup>g</sup>	3.25±0.09 <sup>e</sup>
<i>T. scabrum</i>	95.97±0.14 <sup>b</sup>	9.27±0.04 <sup>b</sup>	21.23±0.11 <sup>bc</sup>	39.40±0.14 <sup>d</sup>	27.38±0.14 <sup>de</sup>	3.90±0.08 <sup>cde</sup>
<i>T. spumosum</i>	96.22±0.08 <sup>ab</sup>	8.60±0.03 <sup>d</sup>	21.00±0.11 <sup>cd</sup>	37.34±1.07 <sup>de</sup>	24.31±0.23 <sup>f</sup>	3.27±0.11 <sup>e</sup>
<i>T. tomentosum</i>	96.28±0.05 <sup>ab</sup>	9.04±0.01 <sup>bc</sup>	20.43±0.12 <sup>d</sup>	33.95±0.40 <sup>ef</sup>	23.15±0.12 <sup>f</sup>	4.18±0.07 <sup>cd</sup>

DM: Dry matter, CA: Crude ash, CP: Crude protein, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin

<sup>a-f</sup>Data with different letters in the same column are different from each other.

CA contents were determined between 5.36% and 9.85% in clover species. While the highest CA content was determined in *T. hybridum*, the lowest was determined in *T. lappaceum*. CA contents varied significantly among the species. As a matter of fact, many researchers have reported that plant mineral matter contents vary significantly among the species.<sup>15-18</sup> CP contents of clover species varied between 18.47 and 22.05% and these values are quite good in terms of nutrient content. The highest CP content was found in *T. resupinatum* while the lowest CP content was found in *T. lappaceum*. It was determined that there was no statistical difference between *T. resupinatum* and *T. hybridum* species. Tekeli and Ates<sup>19</sup> found in their study that CP contents of some clover species vary from species to species and they determined that CP contents varied between 19.01 and 24.51%. As a matter of fact, the CP content results obtained from this study were similar to the results of Tekeli and Ates.<sup>19</sup> Among the clover species, NDF contents varied between 30.31 and 49.80%, the highest value was determined in *T. cherleri* and the lowest in *T. resupinatum*. *T. cherleri* and *T. lappaceum* species were placed statistically in the same group. Similarly, *T.*

*resupinatum* and *T. tomentosum* species were also included in statistically similar groups. Pereira and co-workers<sup>20</sup> studied in some clover species with 316 samples and as a result of this study, they determined that the NDF contents were between 14.54 and 51.58%. The NDF contents obtained from this study were within the ranges reported by Pereira-Crespo.<sup>20</sup> ADF contents of clover species were found to be between 21.32% and 34.28%. Similar to NDF contents, the highest and lowest values in ADF contents were determined in *T. cherleri* and *T. resupinatum* species, respectively. Pereira and co-workers<sup>20</sup> found the ADF contents of some clover species to be between 11.55 and 44.35%, which supports the ADF results obtained from our study. ADL contents of clover species varied between 3.25 and 5.04%. The highest ADL content was determined in *T. hybridum* and the lowest in *T. resupinatum* species. High lignin content is an undesirable feature in feeds<sup>21</sup> and in this respect, the *T. resupinatum* species came to the fore.

Data on Hcel, Cel, DMD, DMI, RFV and NEL characteristics of some clover species and mean comparison test results were given in Table 3.

**Table 3.** Mean comparison test results of Hcel, Cel, DMD, DMI, RFV and NEL traits in some clover species.

Clover species	Hcel %	Cel %	DMD %	DMI %	RFV	NEL %
<i>T. angustifolium</i>	13.00±0.31 <sup>ab</sup>	26.17±0.25 <sup>c</sup>	64.98±0.18 <sup>e</sup>	2.75±0.00 <sup>de</sup>	138.27±0.19 <sup>d</sup>	1.50±0.01 <sup>e</sup>
<i>T. cherleri</i>	15.53±0.44 <sup>a</sup>	30.38±0.38 <sup>a</sup>	62.20±0.26 <sup>e</sup>	2.41±0.04 <sup>e</sup>	116.20±2.11 <sup>e</sup>	1.40±0.01 <sup>g</sup>
<i>T. hybridum</i>	10.53±1.31 <sup>bc</sup>	22.63±0.28 <sup>de</sup>	67.03±0.27 <sup>d</sup>	3.12±0.12 <sup>c</sup>	162.09±6.95 <sup>c</sup>	1.57±0.01 <sup>d</sup>
<i>T. lappaceum</i>	15.97±0.34 <sup>a</sup>	28.96±0.14 <sup>b</sup>	63.51±0.25 <sup>f</sup>	2.47±0.00 <sup>e</sup>	121.63±0.51 <sup>de</sup>	1.44±0.01 <sup>f</sup>
<i>T. nigrescens</i>	11.09±0.41 <sup>bc</sup>	21.81±0.10 <sup>ef</sup>	68.49±0.10 <sup>c</sup>	3.22±0.03 <sup>bc</sup>	170.85±1.75 <sup>c</sup>	1.62±0.00 <sup>c</sup>
<i>T. pilulare</i>	13.69±0.67 <sup>ab</sup>	26.64±0.26 <sup>c</sup>	64.22±0.15 <sup>ef</sup>	2.65±0.03 <sup>e</sup>	131.65±1.16 <sup>de</sup>	1.47±0.01 <sup>ef</sup>
<i>T. resupinatum</i>	8.99±0.70 <sup>c</sup>	18.07±0.18 <sup>g</sup>	72.29±0.13 <sup>a</sup>	3.97±0.11 <sup>a</sup>	222.18±6.41 <sup>a</sup>	1.74±0.01 <sup>a</sup>
<i>T. scabrum</i>	12.02±0.06 <sup>bc</sup>	23.48±0.14 <sup>d</sup>	67.57±0.10 <sup>cd</sup>	3.04±0.01 <sup>cd</sup>	159.48±0.78 <sup>c</sup>	1.58±0.00 <sup>cd</sup>
<i>T. spumosum</i>	13.03±0.92 <sup>ab</sup>	21.05±0.20 <sup>f</sup>	69.96±0.17 <sup>b</sup>	3.22±0.09 <sup>bc</sup>	174.56±5.23 <sup>c</sup>	1.67±0.01 <sup>b</sup>
<i>T. tomentosum</i>	10.80±0.36 <sup>bc</sup>	18.97±0.19 <sup>g</sup>	70.87±0.10 <sup>b</sup>	3.54±0.04 <sup>b</sup>	194.20±2.38 <sup>b</sup>	1.69±0.00 <sup>b</sup>

Hcel: Hemicellulose, Cel: Cellulose, DMD: Dry matter digestibility, DMI: Dry matter intake, RFV: Relative feed value, NEL: Net energy lactation

<sup>a-g</sup>Data with different letters in the same column are different from each other.

Hcel contents of clover species were determined between 8.99 and 15.97%. The highest Hcel content was found in *T. lappaceum* and the lowest in *T. resupinatum*. High Hcel content increases the daily dry matter intake in cows and negatively affects digestibility.<sup>22,23</sup> Cel content of clover species was determined between 18.07 and 30.38%. The highest Cel content was determined in *T. cherleri* and the lowest in *T. resupinatum*. Tekeli and Ates<sup>19</sup> determined the crude fiber contents of clover species in a study they conducted, similar to the results obtained in this study. DMD, DMI, RFV and NEL values of clover species varied between 62.20 and 72.29%, 2.41 and 3.97%, 116.20 and 222.18 and 1.40 and 1.74%, respectively. In terms of these properties, the highest value was obtained from *T. resupinatum* and the lowest from *T. cherleri* species. If the RFV value calculated based on the DMD and DMI characteristics is above 150, the feed is said to be of the best quality.<sup>24</sup> The DMD, DMI and RFV values obtained from this study were similar to the results of Gürsoy and Macit<sup>25</sup> studying with some pasture legumes. Therefore, it was determined that very good quality roughage can be obtained from other clover species except *T. cherleri*, *T. lappaceum* and *T. pilulare*. In addition, *T. resupinatum* was statistically significantly separated by RFV. It was determined that the highest energy can be obtained from *T. resupinatum* in the lactation period of dairy cows. *T. resupinatum* was leading in terms of many features examined.

### 3. CONCLUSION

This study was carried out to compare the chemical composition and nutritional value of some clover species, especially grown in Mediterranean pastures. According to the results of this study, *T. resupinatum* was found to be superior in many features. However, when the results obtained from the study were evaluated collectively, it was determined that *T. resupinatum* and *T. hybridum* offered a superior chemical composition and nutritional value compared to other species. *T. cherleri*, *T. lappaceum* and *T. pilulare* species were found to provide lower quality roughage compared to other species. As a result, it can be said that a pasture dominated by *T. resupinatum* and *T. hybridum* species can provide better quality roughage compared to other species.

### Conflict of interest

I declare that there is no a conflict of interest with any person, institute, company, etc.

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