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Research Article

Determination of *In Vitro* True Digestibility and Relative Feed Values of Alternative Roughage Sources

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Abstract: The aim of this study was to compare chemical composition, relative feed values, and in vitro true digestibility of the giant fennel (*Ferula communicus* L. -F), helis (*Prangos ferulacea* L. -H), gum- tragacanth (*Astragalus microcephalus WILD*. -G) and leaves oak tree (*Quercus robur* L. -O), a naturally growing plant on the high- altitude plateaus of the Eastern Anatolia, with those of alfalfa. In vitro true digestibilities (IVTD) of roughages were determined with the Ankom Daisy[II] incubator. In terms of crude protein (CP) content, the lowest value was determined in H herb with 7.35 %, and the highest value was determined in alfalfa hay (A) with 19.28% ($p<0.05$). G hay had higher acid detergent fiber (ADF) and neutral detergent fiber (NDF) content and O leaves had higher ether extract (EE) and condensed tannin (CT) content. While the lowest IVDMD were found in G hay (42.91%) and O leaves (56.22%) with the highest cell wall structural components and CT content, the highest digestibility value was determined for F (70.47%) and A (71.60%) ($p<0.05$). Considering the analyzed parameters, it can be said that F hay is more suitable for ruminant feeding than other roughages.

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1. Introduction

Livestock animals make critical and valued contributions to society throughout the world and play a key role in agriculture. The main role of ruminants in the ecosystem is the ability to break down the structural carbohydrates in plants and metabolize them to make meat and milk that can be consumed by humans or other animals in the food chain. Roughage is an important main component of ruminants' feeds. Roughage is necessary to keep the maintenance of rumen health as it should. Considering the existence of animals in our country, there is a large deficit in roughage production in terms of both quantity and quality.

According to official data of TUIK (2021a;b), Türkiye has 18 million 26 thousand bovine animal unit livestock existence, and 71.3 million tons of quality roughage is needed to feed the animal existence. However, the total quality roughage production in Türkiye is 67.2 million, 11.7 million from the meadow and grassland lands, and 55.5 million from forage crops. Accordingly, the roughage gap in the country is 4.1 million tons. Ruminant producers are forced to look at alternative sources of roughage to see their animals due to a shortage of roughage.

Ferula communis L. (giant fennel-locally called çakşır or kerkol) and *Prangos ferulacea* L. (locally called helis) is a naturally growing plants in the high- altitude plateaus of the Eastern Anatolian region. This plant produces seeds every other year, but it is not cultivated yet. It is a perennial plant and is most densely diversified in Iran-Turan phytogeographic region (Duran et al., 2005; Hakan et al., 2009; Çelikezen et al., 2019). It is claimed to be effective on oustrus and ovulation of sheep and goats (Keskin et al., 2004; Önal et al., 2004). It is reported that this effect is caused by a phytoestrogenic substance called ferutin (Appendino et al., 2001). Helis is used as hay and winter fodder for ruminant animals in Turkiye, Iran, Central Asia, North India, and the Caucasus (Razavi, 2012). Because it grows on the steep slopes of the mountains, it is mostly evaluated by sheep. Caksir, helis, gum-tragacanth, and oak tree leaves, which grow in the high plateaus of the Eastern Anatolia Region, are harvested in June-July, dried, and used as a source of roughage in winter. Azarfard (2008) suggested that alfalfa hay can be replaced with *P. ferulacea* in the finishing rations of lambs at 35% to 60% levels, while 100% levels increased the fat in the carcass and tail of lambs.

Astragalus species have anti-inflammatory (Kim et al., 2013), immunostimulatory (Qin et al., 2012), antioxidant (Kim and Yang, 2005), and antiviral activities (Sanpha et al., 2013). Due to these properties, it has been reported that Astragalus species are used in the roughage ration, and their by-products are used as additives to the feed of farm animals (Qiao et al., 2018). *Quercus robur* L. is the most common oak species in the forest areas of the Eastern Anatolia Region, where winters are colder and longer, rainfall is heavy, and temperature differences are high. *Quercus robur* L. and *Quercus brandii* L. are common in Hakkari, Bitlis, Muş, Bingöl, Elazığ and Malatya (Günal, 2013). In these regions, oak leaves are an important source of roughage for small ruminants, especially goats. It has been reported that the leaves of trees and shrubs have superior mineral and protein composition compared to some hay and can be used to meet the protein and mineral needs of ruminants which can make good use of low-quality roughage (Kongmanila, 2012).

In this study was compared with traditionally used alfalfa hay of relative feed values and *in vitro* true digestibility of giant fennel, helis, gum-tragacanth, and oak tree leaves, which are widely grown in the high-altitude plateaus of the Eastern Anatolia Region and used by the local people for small ruminants feeding.

2. Material and Methods

2.1. Ethical statement

This study was approved by the Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee (Approval no: 2019/07).

2.2. Plant samples

In the experiment *F. communis* L., *P. ferulacea* L., *A. microcephalus WILD*. and *Q. robur* L. leaves, which are grown in the meadow-pasture and forest areas of Bitlis Province Hizan District and are widely used by the people of the region for feeding small ruminants, were used as feed materials in the experiment. In addition, alfalfa hay (*Medicago sativa* L.), which is traditionally used in ruminant feeding, was compared with other roughages used.

The forages were harvested and dried from the meadow-pasture and forest areas of the villages of Hizan district of Bitlis province in July (giant fennel, helis, and gum-tragacanth samples) and August (oak tree leaves). Plant sampling was done from 3 villages representing Bitlis Province Hizan District and 3 samples from each village. Villages were taken as replicates. All plant samples in the same village were combined. The giant fennel, helis, and gum-tragacanth samples were taken from all parts of the plant, while leaf samples were taken from the oak tree. Plant samples dried in laboratory conditions were ground on a mill and sieved in 1 mm diameter sieve to make the samples ready for chemical analysis.

2.3. Chemical analysis

Dry matter (DM), crude protein (CP) and ash of all the samples were determined according to the standard methods of AOAC (1998). Ether extract (EE) analysis was carried out as described by AOCS Am 5-04 (Komarek et al., 2004). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the methods of Van Soest et al. (1991) using an ANKOM 220 Fiber

Analyzer (ANKOM Technology Corporation, NY, USA). Condensed tannin (CT) was determined by butanol-HCl-iron method as described by Bate-Smith (1975). All chemical analyses were carried out in quadruplicate. The CT (%) of samples was calculated using equations as follows:

$$CT (\%) = Absorbance (550 nm \times 156.5 \times dilution factor) / Dry weight (\%)$$

2.4. *In vitro* true digestibilities

In vitro true digestibility (IVTD) was determined in the Ankom Daisy incubator by using the filter bag technique according to Van Soest *et al*. (1991) and Ankom technology (2002) procedures. In the Ankom Daisy incubator, rumen fluid was used as the inoculum source. The rumen content was obtained post-mortem from the rumens of two Simental cattle aged 22 months old that were slaughtered in a commercial abattoir in Van. Animals were fed twice daily with a diet containing grass, hay, and straw (60%) and concentrates (40%). After filtering the rumen fluid through two layers of cheesecloth under CO_2 into a thermos at 39 °C, approximately 100 g of solid rumen content was added and delivered to the laboratory within 20-25 minutes. The Daisy incubator instrument contains 4 cylinder jars. 400 ml of rumen fluid as inoculum and 1600 ml of buffer solution (solution B and A, mixing ratio 1/5 and mixing pH 6.98) and 24 filter bags were placed in each cylinder jars . The jars were closed immediately after bubbling with CO_2 and allowed to incubate for 48 hours.

The IVTD (%) of samples was calculated using equations as follows:

$$IVTD (\%) = 100 - ((W3 - (W1 \times C1)) \times 100)/W2 \quad (1)$$

W1: Weight of filter bag, W2: Weight of sample, W3: Final weight after NDF analysis,
C1: The bag without a sample was also prepared for correction

2.5. Determination of relative feed value and quality *in vitro* true digestibilities

The relative feed value (RFV), which is used as an important tool in forage quality evaluation and marketing, was calculated according to the following equations (Rohweder et al., 1978).

$$\text{Dry matter intake (DMI, live weight \%)} = 120/(NDF\%) \quad (2)$$
$$\text{Dry matter digestibility (DMD, \%)} = 88.9 - (0.779 \times ADF\%)$$
$$RFV (\%) = (DMI \times DMD)/1.29$$

According to the Quality Standard assigned by The Hay Marketing Task Force of the American Forage and Grassland Council, the RFV was evaluated as roughages based on reject <75 (5), 75-86 poor (4), 87-102 fair (3), 103-124 good (2), 125-151 premium (1) and prime >151.

2.6. Statistical analysis

The data obtained from the experiments were analyzed one-way ANOVA completely randomized design (SAS, 2014). The differences between roughages chemical composition, forage quality, and IVDMD were tested by using Duncan's multiple range test.

3. Results

The chemical composition, the cell wall structural elements of roughages, and condensed tannins are presented in Table 1. Accordingly, H and A hay had the lowest DM content than the other roughages, while the other roughages had no significant differences from each other. The highest ash level was found in H ($p<0.05$), followed by A>F>G=O. The CP content of the roughages ranged from 7.35% in the H hay to 19.28% in the A. The A hay, on the other hand, had higher CP content when compared to its other roughages ($p<0.05$). The EE content of roughages ranged from 5.37 to 0.65%, the highest being in O leaves. The NDF content of the roughages ranged from 34.27 to 64.64%, the highest NDF content was found in G with 64.64%, and this value was followed by O with 53.70%. The ADF content of the roughages ranged from 26.39 to 54.31%, the highest being in the G hay and the lowest in the A hay (p <0.05).

The condensed tannin concentration of the roughages was significantly different (p<0.001). The CT concentration ranged between 0.94 to 6.97 DM%. Among the roughages A hay contained significantly (p<0.05) the lowest CT, while O leaves had the highest concentration of CT.

Table 1. Chemical compositions of selected roughages (DM %)

Roughage	DM	Ash	CP	EE	NDF	ADF	CT
F	93.52±0.74^{a}	7.49±0.21^{c}	9.05±0.59^{b}	2.68±0.22^{b}	34.27±1.08^{e}	31.55±1.03^{c}	2.08±0.12^{c}
H	90.92±0.13^{b}	12.28±0.55^{a}	7.35±0.14^{c}	2.74±0.04^{b}	43.16±0.89^{c}	36.37±0.77^{b}	1.85±0.06^{c}
G	93.70±0.15^{a}	6.20±0.15^{d}	8.24±0.48^{bc}	0.65±0.07^{c}	64.64±0.65^{a}	54.51±0.65^{a}	3.12±0.14^{b}
O	93.42±0.30^{a}	6.04±0.30^{d}	9.00±0.51^{b}	5.37±0.27^{a}	53.70±0.91^{b}	35.68±0.79^{b}	6.97±0.28^{a}
A	90.44±0.21^{b}	9.01±0.52^{b}	19.28±0.09^{a}	2.32±0.09^{b}	37.72±0.92^{d}	26.39±059^{d}	0.94±0.03^{d}
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

^{a,b,c} Values within a row with different superscripts differ significantly at p<0.05.
F: *Ferula communicus* L.- caksir, H: *Prangos ferulacea* L. -helis, G: *Astragalus microcephalus WILD*. - gum-tragacanth and O: *Quercus robur* L.- leaves oak tree, A: *Medicago sativa* L.-alfalfa.

The IVDMD, DMD, DMI, RFV, and RFV quality classes of roughages are presented in Table 2. A hay and F hay outperformed H, O leaves, and G hay with respect to IVDMD, DMD, DMI, and RFV (p<0.001). The IVDMD of roughage feeds ranged from 42.81 to 71.60%, being highest for A hay and lowest for G hay (p<0.05). The RFV adopted in marketing and pricing of roughage in many developed countries was found to be 67.06 on average for G hay in this study. According to RFV results, G hay was classified in "5-reject" quality class.

Table 2. Forage quality and IVDMD values of different roughages

Roughage	DMD,%	DMI, BW%	RFV	RFV Quality	IVDMD,%
F	64.32±1.69^{ab}	3.53±0.24^{a}	154.58±5.88^{a}	Prime	70.47±0.38^{a}
H	60.57±1.11^{b}	2.79±0.11^{b}	131.09±7.56^{b}	Premium (1)	64.74±0.39^{b}
G	46.43±1.13^{c}	1.86±0.06^{c}	67.06±3.78^{c}	Reject (5)	42.81±1.05^{d}
O	61.10±0.98^{b}	2.24±0.05^{c}	105.98±3.40^{b}	Good (2)	56.22±0.68^{c}
A	67.96±0.50^{a}	3.02±0.08^{b}	159.35±5.40^{a}	Prime	71.60±0.77^{a}
P value	<0.001	<0.001	<0.001	<0.001	<0.001

^{a,b,c} Values within a row with different superscripts differ significantly at p<0.05.
F: *Ferula communicus* L.- caksir, H: *Prangos ferulacea* L. -helis, G: *Astragalus microcephalus WILD*. - gum-tragacanth and O: *Quercus robur* L.- leaves oak tree, A: *Medicago sativa* L.-alfalfa; DMD: Dry matter digestibility, DMI: Dry matter intake, RFV: Relative feed value, BW: Body weight, IVDMD: *In vitro* dry matter digestibility.

4. Discussion

In this study, it was determined that the dry matter contents of F hay, G hay, and O leaves are higher than A and H hay. As it is known, trees and shrubs have higher DM and fiber concentrations and lower CP concentrations in summer than herbs (Demir and Keskin, 2016; Dökülgen and Temel, 2020). In this study, the CP content of hays ranged from 7.35 to 19.28%. In this study, the CP values found for H hay and G hay were similar to the ones found for *P. ferulacea* L. (3.92-10.1%) and *A. gummifer* L. (6.38-9.66%) (Yurtseven, 2011). The CP content of 19.28% determined in A hay seems to be well above the daily protein requirement of ruminants (NRC, 1996). In addition, the CP content of the ration should not fall below 7% for the ammonia nitrogen level required in the rumen in order for the microbial activity in the rumen to continue in its normal course and for the continuity of the microbial activity (Norton, 1994; Cappellozza, 2013). In this study, it is seen that the roughages contain 7% and more CP. The H hay had the lowest (7.35%) CP ratio among the other roughages used in the study. In ruminant rations, the protein value of the rations can be balanced according to the yield level of ruminants by mixing a little F, H, G, and O leaves together with A hay.

While G hay and O leaves had the lowest (0.65%) and the highest (5.37%) EE content, respectively, F, H, and A hay were found to be similar in terms of EE content. It has been reported that the CF content of shrubs and tree leaves varies according to the season, and is generally low in spring and summer and higher in autumn and winter (Alatürk et al., 2014). In a study, the EE content of Kermes oak leaves in spring and summer was 6.03% and 4.53%, respectively. It has been reported that this rate increases to 8.06% and 8.10% in the summer and autumn months (Alatürk et al., 2014).

The quality of the forage depends on the amount and ratio of fiber in it. This is because fiber is more difficult to digest than the non-fiber components of forage. Also, the rate at which fiber is digested slows as plants mature. While ADF is an indicator of the digestibility of the plant, NDF is a good predictor of ruminant dry matter consumption and gastrointestinal fullness (Van Soest, 1982). The NRC recommendation for NDF in a diet is 30%, and the primary source of a minimum of 21% NDF is roughage. As a general rule, feedstuff which has 11-13% CP are capable of supplying adequate protein for maintenance and growth, while feedstuffs with low NDF (20-25%) are more digestible than those with more than 35% NDF (Norton, 1994). The CP (12.2% and 10.2%), CF (3.4% and 4.7%), and ADF (24.4% and 23.2%) contents reported by Shawrang et al. (2013) for *P. ferulacea* and *F. orientalis* are in the ranges reported for H hay and F hay in the current study.

In an evaluation to be made in terms of condensed tannin as secondary components that prevent the use of nutrient matters in forage, oak tree leaves and gum-tragacanth were the most striking. We found the highest condensed tannin concentration (6.97%) in oak tree leaves. This was followed by gum-tragacanth with 3.12% and caksir with 2.08%. Ataşoğlu et al. (2010) found that condensed tannin content in Kermes oak varies between 5.83% and 13.8% in dry matter, Sevim and Sarı (2014) found that condensed tannin content in kermes oak leaves was 9.61%, Kamalak et al. (2015) 9.22%, Imik (1997) 8.02% and Alatürk et al. (2014) reported that it varies between 17.09 g/kg DM and 19.26 g/kg DM. Examining the phytochemical and biological properties of 4 different gum-tragacanth species commonly used in folk medicine, Jaradat et al. (2017) stated that the total tannin content varies between 12.78 mg TA/g and 22.54 mg TA/g, and its content varies according to gum-tragacanth species. Studying the potential nutritional value of gum-tragacanth species at different maturation periods, Çacan et al. (2017) reported that the condensed tannin content is between 0.47% and 0.78%, and the said values vary according to the gum-tragacanth species and harvest periods. It has been reported that the total tannin content in roots and fruits varies between 3.76% and 5.70% in different *Ferula sp*., and gallic tannin is not found in the mentioned parts (Baytop, 1967). When the condensed tannin content is examined, it is thought that if oak leaves are used alone as a source of forage, feed consumption will decrease (Kamalak, 2007). In addition to these, 6% in the rations of sheep; it has been reported that goats can tolerate the presence of 8-10% tannin in their rations (İmik and Şeker, 1999). However, since goats have a higher tolerance (8-10%) against condensed tannin than sheep, it is estimated that oak leaves with higher condensed tannin content (6.97%) will be more suitable for use in goat rations.

A and F hay mark the highest IVDMD, DMD, and RFV values, while G hay showed the lowest values. The amount of dry matter consumption of the animals is related to the NDF content of the feeds, and the feeds with high NDF content have a lower DMI value (Özcan and Kılıç, 2018). From this point of view, it can be said that the F hay can be consumed more willingly by animals. Jančík et al. (2017) suggested that the *in vitro* digestibility value of alfalfa hay by Daisy II is 79.6%. This value is similar to the value found for alfalfa hay in this study, while Ekinci et al. (2018) are higher than the value of the IVDMD value (62.53 %) found for alfalfa hay. The mean values obtained for IVDMD in the K hay (72.43%) are higher than that obtained by Shawrang et al. (2013) for effective DM degradability (56.0%) of *Ferula orientalis* by nylon bag technique. It is possible to see the differences between the changes in the plant composition during the harvest stage and the methods used to determine the digestibility value.

The H hay marked the high IVDMD, DMD, DMI, and RFV values, while O leaves and G hay showed the lowest values. The reason behind this finding was that G hay and O leaves is poor in terms of carbohydrate and protein contents that can be used by rumen microorganisms while being rich in cell wall structural elements and condensed tannin. Indeed, especially the increase in ADF content adversely affects digestibility. Aldemir et al. (2015) and Coşkun et al. (2004) suggested that the in vitro dry matter digestibility of *Prangos ferulacea* hay is 79.15 and 80.60%, respectively. These values are higher than the values found H hay in this study, while Shawrang et al. (2013) are similar to the effective dry matter degradability value found for *Prangos ferulacea*. Yurtseven (2011) suggested that DMD (55.83% - 66.77%), DMI (2.13- 2.67% BW), and RFV (112.57-115.98) values for helis hay are adversely affected by delayed harvest time.

In the present study, IVDMD, DMI, DMD and RFV values of O leaves were determined to be 56.22%, 2.24%BW, 61.10%, and 105.98, respectively. According to Dökülgen and Temel (2020), the DMD of Kermes oak leaves in the autumn and spring were 57.5% and 69.00%, respectively. This was due to the increase in leaves and shoots in the spring and the lower NDF and ADF contents compared to the autumn months. They also stated that RFV values in autumn (125.09) are lower than in spring months (173.66). Parlak et al. (2011) declared that dry matter digestibility of Kermes oak was the highest in spring (70%) and was between 43.6 to 51.4% in other mounts of the year.

The lowest IVDMD value of 42.81% was determined in the G hay. As RFV falls below 100, feed quality decreases (Richardson, 2020). As seen in Table 1, the highest ADF (54.51%) and NDF values (64.64%) are in G hay. The increase in NDF and ADF causes a decrease in RFV of feeds, and an increase in intracellular components causes an increase in RFV. The value of DMD calculated from the ADF value (46.43%) and the level of DMI calculated using the NDF value (1.86%) was the lowest in G herb. Therefore, it is seen that the values calculated in terms of RFV are in parallel with the *in vitro* digestion values. Yurtseven (2011) determined that the gum-tragacanth plant contains higher ADF and NDF, probably due to its thorny structure, and that the DMD value determined by the gas production technique is lower (37.39%-42.84%) compared to alfalfa hay and helis.

5. Conclusion

As a result, considering the problems in roughage in Türkiye, the use of alternative feed sources such as tree leaves is of particular importance. Considering their nutrient content, it is not possible to use caksir, helis, gum-tragacanth, and oak tree leaves alone as an alternative roughage source to alfalfa hay. It can be recommended to use caksir, helis, gum-tragacanth plant, and oak leaves together with other roughage in the roughage ration, depending on the quality of the roughage. Additional studies, however, are needed to elucidate the longer associative effects of roughage source on rumen fermentation and digestibility in small ruminants.

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