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RESEARCH ARTICLE

Nutrient and Mineral Contents, and *In vitro* Digestibility of Kermes Oak (*Quercus coccifera* L.) and Mock Privet (*Phillyrea latifolia* L.)

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ABSTRACT

This study was conducted to exhibit the importance of kermes oak (*Quercus coccifera* L.) and mock privet (*Phillyrea latifolia* L.) in the grazing system and animal feeding. For this purpose, the leaves of these two evergreen shrubs were harvested by hand-clipping in their early vegetative stages at Şarköy, Tekirdağ, Turkey, in April 2019. The nutrient and mineral contents and *in vitro* digestibility of kermes oak and mock privet was determined by using *in vitro* gas production technique. In this study, it was found that there was a statistically significant difference between the kermes oak and mock privet in terms of the contents of dry matter, crude ash, crude cellulose, neutral detergent fiber, acid detergent fiber and acid detergent lignin, and the minerals of zinc, copper, iron, and potassium ($p<0.05$). Furthermore, it has been observed that these two shrub plants yielded different values in terms of metabolizable energy and organic matter digestibility ($p<0.05$). In this study, the time-dependent mean gas production of the two evergreen shrubs was found to be significant at all times except the 96th hour; and gas production kinetics made a significant difference in b, the volume of the gas production from slowly fermentable ($p<0.05$). The volume of the gas production from slowly fermentable (b) was found to be higher in the mock privet than the kermes oak. Results derived from this study indicate that even the differences between the kermes oak and mock privet in terms of nutrient and mineral contents, both shrubs might be used more adequately as an alternative feed source during the grazing season where they are widely distributed.

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Introduction

Mediterranean climate is dominant in the Mediterranean, Aegean, and Marmara Regions in Turkey. In this climate, summers are hot and dry; winters are cool and rainy. The dry summer months cause the deciduous plants grown in these areas to go dry. On the other hand, the evergreen plants manage to stay green by means of up taking water from the depths of the soil in dry seasons. Shrubs are the most prominent of these plants. About half of the Mediterranean vegetation consists of shrubs (Yılmaz, 1996). The shrubs cover an area of 100 million hectares in the world; 32 million hectares of them are in the countries close to the Mediterranean. The tall ones are called maquis, and the dwarf ones are called garrigue. Mediterranean shrubs cover an area

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of about 7 million hectares in Turkey (Baytekin et al., 2005). The dwarf shrubs are seen on poor, barren, and shallow soils of the Mediterranean belt. These shrubs are in the tertiary vegetation and have become widespread as a result of the destruction of forest lands and abandonment of the fields (Atalay et al., 2003).

Kermes oak *(Quercus coccifera* L.*)* and mock privet (*Phillyrea latifolia* L.) are frequently seen in the Mediterranean shrubs (Yılmaz, 1996). The shrub (maquis and garrigue) communities with high fiber content are important feed sources for animals. The young shoots and leaves of these species have been found to contain more nutrients than the herbaceous species in their early stages. The nutritive value is very important, especially in summers (Kamalak, 2006; Narvaez et al., 2010; Akbağ et al., 2019). Shrubs fulfill the protein needs of grazing animals to a large extent. Furthermore, they are an important source of roughage in late winter and summer, and especially their seeds are indispensable feeding sources for wildlife animals in winter (Koç, 2000).

In this study, it has been aimed to determine the nutrient composition, mineral content, and digestion degree of kermes oak and mock privet, the indispensable feeding sources of the Mediterranean climate zone shrublands, using *in vitro* gas production technique.

Materials and Methods

Shrub Leaves

The leaves have been harvested from kermes oak (*Q. coccifera* L.) and mock privet (*P. latifolia* L.) in their early vegetative stages at Şarköy (40.7328 N, 27.1485 E), Tekirdağ, Turkey in April 2019. The mean annual rainfall and temperature were about 600 mm and 13.5°C, respectively. The leaves were collected from three different locations in the main sampling area by hand-clipping. In each location, the samples collected at least 20 different shrubs and then pooled to record their fresh weights. The shrub samples were left for drying under the shade for at least four days in the laboratory. While the chemical analyses were carried out in Tekirdağ Namık Kemal University, and the *in vitro* gas production technique was applied in Kastamonu University.

Chemical Analyses

All samples have been milled through a 1 mm sieve after transported to the laboratory and stored in a glass jar for further chemical analysis. The dry matter (DM), crude protein (CP), crude ash (CA), ether extract (EE), and crude fiber (CF) compositions of kermes oak (*Q. coccifera* L.) and mock privet (*P. latifolia* L.) were analyzed using the AOAC methods (AOAC, 1990). The neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents were determined according to the methods reported in Van Soest et al. (1991). For mineral analysis, approximately 0.5 g of sample was weighed into a vessel, resistant to temperature and pressure, with 10 ml nitric acid and digested in CEM Mars 5 microwave digestion system under constant pressure and temperatures (800 psi and 200°C) for 15 minutes. The digested sample was then allowed to cool before being transferred quantitatively into the clean falcon tubes, completed to 25 ml final volume with deionized water, and analyzed with a Varian AA240 flame atomic absorption spectrophotometer (Huang et al., 2004). All the chemical analyses were carried out in triplicate.

In vitro Gas Production

In vitro gas production of kermes oak and mock privet samples were measured using the ANKOM^{RF} gas production system. Twenty ml of particle-free rumen fluid and 80 ml of the buffer solution and 1 g samples were added to each bottle (Goering and Van Soest, 1970), with no trypticase. All the equipment was pre-warmed at 39°C before the injection of a 100 ml rumen fluid-buffer mixture (1:4) into each 250 ml bottles. Three parallels of each sample were used in the *in vitro* gas production experiment. A total of 21 bottles were put in the same incubation set (3 locations × 2 shrubs × 3 parallels and 3 blanks). All the glass bottles containing incubation medium and feed samples were incubated for 0, 3, 6, 12, 24, 48, 72, and 96 h. Total gas values were corrected for blank incubation. These shrubs are preferred mostly by goats; therefore, the rumen fluid used in this study was obtained from the goats freshly slaughtered at a local slaughterhouse and filtered through four layers of cheesecloth into a pre-warmed thermos and immediately transferred to the laboratory within 20 minutes. Anaerobic conditions were maintained throughout the preparation stages of rumen fluid and the conduct of the experiment. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979) by using Solver in MS Excel:

$Y = a + b\ (1\text{-}exp^{-ct})$

where,

Y: gas produced at time t

a: gas production from the immediately soluble fraction (ml)

b: gas production from the insoluble fraction (ml)

c: gas production rate constant for the insoluble fraction (ml/h)

t: incubation time (h)

The metabolizable energy (ME, MJ/kg DM) and organic matter digestibility (OMD, %) of kermes oak and mock privet were estimated from the measured pressure by *in vitro* gas production method at 24 h by using the following equations of Menke and Steingass (1988) as follows:

$ME\ (MJ/kg\ DM) = 2.20 + 0.1357xGP + 0.057xCP + 0.002859xEE^2$

$OMD\ (\%) = 14.88 + 0.8893xGP + 0.448xCP + 0.651xCA$

Statistical Analysis

One-way analysis of variance (ANOVA) was performed using SAS (JMP, Version 13.2) to determine the effects of kermes oak and mock privet on the nutritive value, selected mineral concentration, and *in vitro* gas production. The significance between the individual means was identified using the t-Test. Mean differences were considered significant at $p<0.05$.

Results and Discussion

In this study, kermes oak and mock privet were examined in terms of the nutrient composition, selected mineral levels, ME, OMD, and *in vitro* gas production parameters. The nutrient analyses and mineral contents of the kermes oak and mock privet were given in Table 1. The average gas productions at different times of treatments and the *in vitro* gas production kinetics of treatment groups after 96 hours of incubation were given in Tables 2 and 3, respectively. The relationship between the *in vitro* gas production and the incubation time in kermes oak and mock privet was given in Figure 1.

Table 1. Nutrient composition of kermes oak and mock privet

	Kermes oak (*Q. coccifera*)	Mock privet (*P. latifolia)*	SEM[x]	Sig.
DM, g/kg	299.0[b]	317.6[a]	4.63	*
CP, g/kg	85.6	92.2	2.20	NS
EE, g/kg	38.5	38.5	2.3	NS
CF, g/kg	297.6[a]	226.1[b]	4.0	***
CA, g/kg	30.1[b]	34.3a	0.3	***
NDF, g/kg	471.5[a]	420.2[b]	3.5	**
ADF, g/kg	367.4[a]	321.3[b]	5.0	**
ADL, g/kg	163.1[b]	192.8[a]	3.7	**
Zn (mg/kg)	17.07[b]	37.83[a]	0.08	***
Cu (mg/kg)	6.32[b]	7.76[a]	0.14	**
Fe (mg/kg)	161.30[a]	133.41[b]	2.70	**
Na (g/kg)	0.97	1.05	0.15	NS
K (g/kg)	9.60[b]	15.04[a]	0.25	***
Pb (mg/kg)	2.76	5.22	0.94	NS
Cd (mg/kg)	0.68	0.67	0.02	NS
ME (MJ/kg DM)	9.22[b]	11.38[a]	0.36	*
OMD (%)	61.42[b]	75.61[a]	2.36	*

[x]Standard error of mean uses a pooled estimate of error variance.
a, b: The values with different letters in the same row differ significantly.
SEM: Standard error of mean, NS: Not significant, *p<0.05, **p<0.01, ***p<0.001.

Table 2. Average gas production at different times of *in vitro* incubations

	3h (ml)	6h (ml)	12h (ml)	24h (ml)	48h (ml)	72h (ml)	96h (ml)
Kermes oak (Q. coccifera)	20.11[b]	27.84[b]	39.93[b]	47.89[b]	66.63[b]	74.46[b]	69.64
Mock privet (P. latifolia)	24.38[a]	36.16[a]	51.45[a]	63.45[a]	86.15[a]	90.02[a]	84.15
SEM[x]	0.78	1.30	1.53	2.68	3.58	3.80	4.29
Sig.	*	*	**	*	*	*	NS

[x]Standard error of mean uses a pooled estimate of error variance.
a, b: The values with different letters in the same column differ significantly.
SEM: Standard error of the mean, NS: Not significant, *p<0.05, **p<0.01.

Table 3. *In vitro* gas production kinetics of shrub samples after 96 hours of incubation

	c	a	b	a+b
Kermes oak (Q. coccifera)	0.0565	5.45	66.01[b]	71.45
Mock privet (P. latifolia)	0.0700	4.75	82.17[a]	86.92
SEM[x]	0.005	0.555	3.91	4.19
Sig.	NS	NS	*	NS

[x]Standard error of mean uses a pooled estimate of error variance.
a, b: The values with different letters in the same column differ significantly.
SEM: Standard error of the mean, NS: Not significant, *p<0.05, **p<0.01.

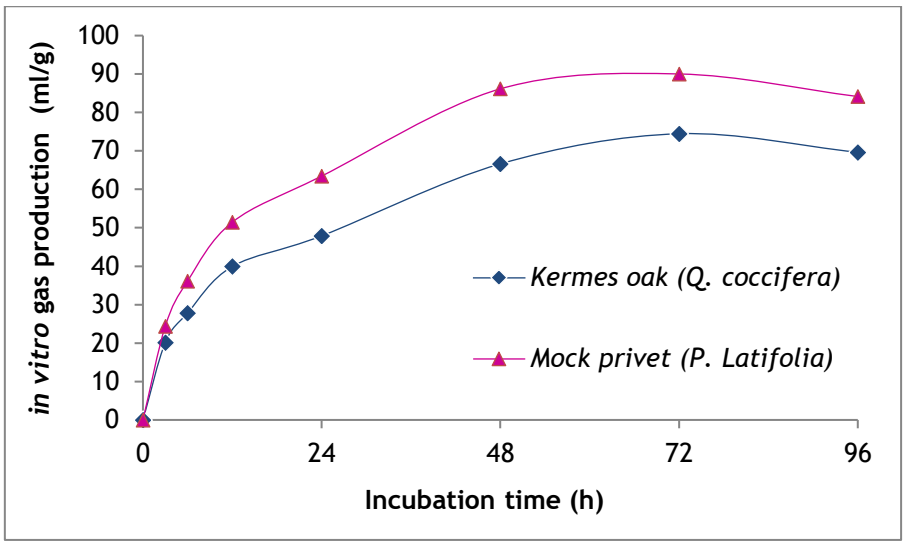

Figure 1. The relationship between *in vitro* gas production and incubation time in kermes oak (*Q. coccifera* L.) and mock privet (*P. latifolia* L.)

When the Table 1 showing the nutrient analyses of the kermes oak and the mock privet was examined, it was found that there was a statistically significant difference between the two maquis species in terms of the DM ($p<0.05$), CF ($p<0.001$), CA ($p<0.001$), NDF, ADF, and ADL contents ($p<0.01$).

In this study, the DM contents of kermes oak and mock privet varied between 299.0 and 317.6 g/kg; and the DM content of mock privet was found to be higher. In their study monitoring the DM content of the kermes oak on a monthly basis in the vegetation period, Tolunay et al. (2009) asserted that the DM contents in April and September were 320.3 g/kg and 579.5 g/kg, respectively; and the DM content increased as the months progressed. In another study by Yolcu et al. (2014), it was reported that the DM contents of kermes oak and mock privet showed variation depending on the month (April, June, and September); and in April, the DM content of the mock privet (336.8 g/kg) was higher than that of the kermes oak (289.3 g/kg). Türel and Buğdaycı (2020) reported that the DM content of the kermes oak in Burdur varied depending on the season; the highest DM content was observed in winter, and the mean DM content was 571.2 g/kg.

When the kermes oak and mock privet were compared in terms of CP, it was observed that both shrub plants yielded similar values (85.6-92.2 g/kg) ($p>0.05$). Parlak et al. (2011) reported the CP content of the kermes oak as 76.7 g/kg. In a study examining the change in the CP content depending on the seasons, the CP content of kermes oak was found to be the highest in winter and spring (64.3 g/kg) and the lowest in summer (59.3 g/kg); on the other hand, the mock privet yielded the highest CP content in spring (Alatürk et al., 2014). In the same study, likewise the finding we reached in our study, the mean CP content in mock privet (70.2 g/kg), was found to be higher than the kermes oak (62.8 g/kg). Roukos (2014) asserted that the CP content in kermes oak leaves varied depending on season and altitude, and it was 103.0 g/kg at low altitude, 105.0 g/kg at mid-altitude, and 106.0 g/kg at high altitude. Kamalak et al. (2015) reported that the CP contents in kermes oak leaves and acorns were 91.7 g/kg and 42.3 g/kg, respectively. Türel and Buğdaycı (2020) asserted that CP contents in the kermes oak varied depending on the season, and the CP contents in fall, winter, spring, and summer were 75.8, 75.1, 71.6 and 69.3 g/kg, respectively.

The variance observed in the CP results of kermes oaks in different studies may stem from the difference of varieties or lines used, different regions, analysis methods, processing techniques, and harvest times.

In our study, the ether extracts (EE) contents of both shrub plants were found to be 38.5 g/kg ($p>0.05$), which is kermes oak = mock privet in terms of EE content. While Alatürk et al. (2014) found that the EE content of the mock privet (75.7 g/kg) was higher than that of the kermes oak (66.8 g/kg); Yolcu et al. (2014) found that the EE contents varied depending on the months, and the EE contents in different months were 14.6, 24.9, and 56.9 g/kg in kermes oak and 18.3, 20.1, and 21.8 g/kg in mock privet. Türel and Buğdaycı (2020) also reported that the EE contents of kermes oak varied depending on the season and altitude within the range of 30.4-55.4 g/kg.

The crude fiber (CF) contents of the 2 shrub plants being examined in this study created a statistically significant difference between the species ($p<0.001$), with the CF content being 297.6 g/kg in kermes oak and 226.1 g/kg in mock privet. In another study, it was found that the CF contents of kermes oak (124.2, 289.8, and 312.8 g/kg, respectively) were higher than those of the mock privet (116.0, 240.8 and 189.2 g/kg, respectively) in April, June, and September (Yolcu et al., 2014). In another study, the CF content in kermes oak was found to be within the range of 210.0-233.7 g (Türel and Buğdaycı, 2020).

The CA is calculated by proportioning the ash obtained by burning the sample at 550°C to the initial amount of the sample and expressed in percentage; in our study, the CA content varied between 30.1 g/kg and 34.3 g/kg ($p<0.05$) and it was found to be higher in the mock privet than the kermes

oak. In a study, it was reported that the CA content in the kermes oak was within the range of 15.4-23.7 g/kg (Tolunay et al., 2009). Kökten et al. (2012) found the CA contents in kermes oak and mock privet as 42.0 and 32.0 g/kg, respectively. On the other hand, Türel and Buğdaycı (2020) found the CA content to be 40.5 g/kg in the kermes oak. Whereas the amount of ME in the plant material is inversely associated with cell wall components, it has a positive association with CP and ash.

The NDF, which contains the hemicellulose, cellulose, and lignin, led to a statistically significant difference ($p<0.01$) between the plants, and the NDF contents were found to be as follows: Kermes oak (471.5 g/kg) > mock privet (420.2 g/kg). The fibrous compounds that make up the cell wall are NDF, ADF, and ADL. The ADF, containing cellulose and lignin, caused a statistically significant difference ($p<0.01$) between the plants. The ADL, a compound affecting animal's ability to digest the grass, was found to be 163.1 g/kg in kermes oak, 192.8 g/kg in mock privet, which revealed a statistically significant difference ($p<0.01$).

Reporting that the NDF content varied depending on the plant species and seasons, Alatürk et al. (2014) asserted that the NDF content in the kermes oak (524.7 g/kg) was found to be higher than the mock privet (477.7 g/kg). In the same study, likewise, in the NDF contents, the ADF contents were also found to be higher in the kermes oak (342.2 g/kg) than the mock privet (335.3 g/kg). Unlike the findings of our study, Alatürk et al. (2014) asserted that the ADL content, which refers to the level of lignin, the indigestible part of the plant, in kermes oak (146.9 g/kg) was higher than that in the mock privet (129.8 g/kg). This difference may stem from the differences in terms of cultivation regions, harvesting methods, seasons, and analysis methods.

Parlak et al. (2011) reported that the NDF, ADF, and ADL contents in kermes oak were 568.4, 443.5, 191.6 g/kg, respectively. In another study, the NDF and ADF contents were found to vary depending on the altitude of the region where the kermes oak was grown, and it was reported that the NDF content was 477.0 g/kg at low altitude, 492.0 g/kg at mid-altitude, and 455.0 g/kg at high altitude (Roukos, 2014). In the same study, the ADF contents were found to be 312.0, 297.0, 298.0 g/kg, respectively. Türel and Buğdaycı (2020) found the NDF, ADF, and ADL contents in the kermes oak to be within the ranges of 477.1-692.8, 476.2-641.3, and 277.2-341.4 g/kg, respectively. The values they found for all 3 parameters were higher than those we found in the study. This may stem from the differences in terms of the part of the plant; the sample is taken from cultivation and harvesting regions, analysis methods, seasons, and altitude.

The comparison between the kermes oak and mock privet in terms of Zn content revealed that the mock privet had a higher Zn content than the kermes oak, and the Zn contents were within the range of 17.07-37.83 mg/kg. In a study, it was reported that the Zn content in the trunk of kermes oak varied within the range of 3.00-5.90 mg/kg depending on the altitude (Roukos et al., 2017).

The two maquis shrubs yielded different cupper (Cu) contents. The Cu content in shrubs varies depending on the plant species, maturity period, season, and soil characteristics (Gökkuş et al., 2013). In a study, it was reported that the Cu content in the kermes oak differed depending on the altitude and was different in the trunk and acorn, and the Cu content in the kermes oak acorn varied within the range of 1.60-2.80 mg/kg depending on the altitude (Roukos et al., 2017). The iron (Fe) content was found to be 161.30 mg/kg in kermes oak and 133.4 mg/kg in mock privet ($p<0.01$). In a study, it was reported that the Fe content in the trunk of kermes oak varied within the range of 22.41-29.36 mg/kg depending on the altitude (Roukos et al., 2017).

Being insignificant in terms of sodium (Na), lead (Pb), and cadmium (Cd), the maquis plants created a statistically significant difference in terms of potassium (K) in our study. Potassium also plays an active role in the regulation of osmotic balance in plants. In our study, mock privet and kermes oak were found to contain 9.60-15.04 g/kg K, respectively. In a study, it was reported that the mineral contents in the kermes oak and mock privet varied depending on the months, and the mean K concentrations were found to be 6.85 g/kg and 8.64 g/kg DM in kermes oak and mock privet, respectively (Gökkuş et al., 2011). Potassium is inversely proportional to NDF. In our study, whereas the NDF was found to be high in kermes oak, the K content was found to be low. Roukos et al. (2017) reported that the K content in the acorn of kermes oak varied within the range of 2.83-3.17 mg/kg, depending on the altitude.

The ME contents of kermes oak and mock privet ranged from 9.22 to 11.38 MJ/kg DM, and the ME content of the mock privet was found to be higher than the kermes oak ($p<0.05$). Parlak et al. (2011) reported that the ME content in the kermes oak varied depending on the months, and the mean ME level was 1.99 Mcal/g. Akbağ (2013) also reported that there was a variation in the ME contents between March and October, and it was 8.99-9.53 MJ/kg DM in the kermes oak and 10.65-14.74 MJ/kg DM in mock privet. Roukos (2014) reported that the content of ME in the kermes oak leaves differed depending on the months and altitudes, and it was 8.24 MJ/kg at low altitude, 8.28 MJ/kg at mid-altitude, and 8.61 MJ/kg at high altitude. Supporting our finding, Yolcu et al. (2014) asserted that the ME level in mock privet was higher than that in the kermes oak in April, June, and September. On the other hand, Akbağ et al. (2019) reported that the ME content was 1.99 Mcal/kg DM in the kermes oak and 2.9 Mcal/kg DM in the mock privet.

OMD was found to be 61.42% in the kermes oak and 75.61% in the mock privet ($p<0.05$). Tolunay et al. (2009) asserted that the OMD level in the kermes oak ranged between 30.49% and 56.07% depending on the vegetation period. On the other hand, Yolcu et al. (2014) asserted that the OMD level in mock privet was higher than that in the kermes oak in April and September.

When the Table 2 showing the mean gas production of different maquis plants at different times was examined, it was observed that the mean gas productions created a

statistically significant difference ($p<0.05$) in both maquis shrubs; and the mean gas production in 96 hours was found to be 69.64 ml in kermes oak and 84.15 ml in mock privet. In comparison, the mean gas production was observed to be statistically significant between the 3rd and 72nd hour ($p<0.05$); it was found to be statistically insignificant only at 96th hour. In a study on this subject, it was reported that the gas production at the end of 96 hours varied depending on the months in the mock privet and the kermes oak; and the gas productions were found to be 30.07 ml and 40.50 ml in the kermes oak and mock privet, respectively (Akbağ, 2013). This supports the finding we obtained in this study.

Whereas the *in vitro* gas production kinetics of the kermes oak and mock privet after a 96-hour-incubation made no significant difference in the volume of gas (ml) formed at the moment when the feed was put into the artificial rumen (a), the gas production rate constant (c), and the total (potential) gas production (a+b); it made a significant difference in b, the volume of the gas formed depending on time ($p<0.05$). The volume of the gas formed depending on time (b; refers to as the gas production from the slowly fermentable or insoluble fractions) was found to be higher in the mock privet than the kermes oak. Ataşoğlu et al. (2010) asserted that the digestibility of the kermes oak leaf varied depending on the growth period, climate condition, soil condition, and drying methods.

Conclusion

In this study, it was aimed to determine the nutrient and mineral contents, and *in vitro* digestibility of kermes oak *(Q. coccifera* L.) and mock privet *(P. latifolia* L.) using *in vitro* gas production technique. Although, there was a statistically significant difference between the kermes oak and mock privet in terms of DM, CA, CC, NDF, ADF, ADL, and the minerals of Zn, Cu, Fe, and K contents ($p<0.05$); these two evergreen shrubs might be used more adequately as an alternative feed source during the grazing season where they are widely distributed.

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