Effect of Concentrate and Polyethylene Glycol Supplementation on *In vitro* Gas Production Characteristics of Some Shrub Species

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Abstract

This study aims to determine the chemical composition and *in vitro* fermentation characteristics of some shrub leaves (*Quercus coccifera L., Phillyrea latifolia L., Ephedra major L., Spartium junceum L.*) at different sampling periods and to determine the effect of polyethylene glycol (PEG), and concentrate feed (CT) supplementation on fermentation kinetics in *in vitro* incubations. Shrub samples were harvested in March, April, June, July, September and October. The chemical composition and *in vitro* fermentation characteristics of the shrub species were determined. Furthermore, the nutritive value of shrub species was estimated with the requirements for model goat's maintenance and lactation periods regarding energy and protein concentration. As a results of this study dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF) and total phenolic compounds (TP) concentration differed significantly among sampling periods ($P<0.05$). The cumulative gas production of *Quercus coccifera L.*, *Phillyrea latifolia L.* and *Ephedra major L.* was significantly changed by sampling periods ($P <0.05$). The PEG treatments significantly increased the cumulative gas production in *Quercus coccifera L.* and *Ephedra major L.* ($P <0.05$). The CT treatments significantly increased cumulative gas production in *Ephedra major L.* ($P <0.05$). In conclusion, it is thought that the shrub species that are the subject of this study will not be adequate to meet the nutrient needs of a high-yielding goat, and supplementary with protein-rich feeding, will be needed.
Keywords: goat, tannin, phenolic compounds, gas production

In Vitro İnkübasyonlarda Konsantre Yem ve Polietilen Glikol İlavesinin Bazı Çalı Türlerinin Rumen Fermentasyon Özellikleri Üzerine Etkileri

Öz

Bu çalışmanın amacını *Quercus coccifera L., Phillyrea latifolia L., Ephedra major L.* ve *Spartium junceum L.* çalılarından farklı örnekleme dönemlerinde alınan yaprak örneklerinin kimyasal bileşimini ve *in vitro* fermantasyon özelliklerini belirlemek ve *in vitro* inkübasyonlara polietilen glikol (PEG) ve konsantre yem (CT) ilavesinin fermantasyon kinetiği üzerine olan etkisini belirlemek oluşturmuştur. Bu amaçla çalı örnekleri Mart, Nisan, Haziran, Temmuz, Eylül ve Ekim aylarında toplanarak, kimyasal bileşim ve *in vitro* fermantasyon özellikleri belirlendi. Ayrıca, çalı türlerinin besleme değerinin ortaya konması açısından model hayvan olarak seçilen bir keçinin yaşama payı ve laktasyon dönemi enerji ve protein gereksinim konsantrasyonları açısından tahmin edildi. Çalı örneklerinin kuru madde (KM), nötral çözücülerde çözünmeyen karbonhidrat (NDF), asit çözücülerde çözünmeyen karbonhidrat (ADF) ve toplam fenolik bileşen (TP) içerikleri örnekleme dönemleri arasında önemli ölçüde farklılık gösterdi ($P<0.05$). *Quercus coccifera L., Phillyrea latifolia L.* ve *Ephedra major L.*'un kümülatif gaz üretimi, örnekleme periyotları ile önemli ölçüde değişmiştir ($P<0.05$). *In vitro* inkübasyonlara PEG ilavesi, *Quercus coccifera L.* ve *Ephedra major L.*'un kümülatif gaz üretimini önemli ölçüde artırdığı belirlenmiştir ($P<0.05$). *In vitro* inkübasyonlara CT ilavesi ile *Ephedra major L.*'un kümülatif gaz üretiminin önemli ölçüde arttığı bulgulanmıştır ($P <0.05$). Sonuç olarak bu çalışmaya konu olan çalı türlerinin, yüksek verime sahip bir keçinin besin madde ihtiyaçlarını karşılamada yetersiz kalacağı ve özellikle proteince zengin ek yemlemeye ihtiyaç duyulacağı düşünülmektedir.
Anahtar Kelimeler: keçi, tanen, fenolik bileşen, gaz üretimi

Introduction

Shrubby vegetation is an important component of the ecosystem due to its resistance to extreme climatic conditions, provides high-quality feed for animals and their role in the stability and sustainability of the ecosystem, shrubs protect the herbaceous species from grazing pressure of herbivores and contribute to the rehabilitation of marginal lands (El Aich, 1991). Thanks to the shrubby vegetation, lots of seeds can germinate which contributes to the diversification of these grazing lands (Özaslan-Parlak et al., 2011). Furthermore, shrublands provide habitats for wildlife animals (Papachristou et al., 2003) an important source of goat feed throughout the year (Perevolotsky et al., 1998). In general, the nutritive value of these browsing lands is variable (Rogosic et al., 2006) and the nutritive value of shrubs for goats are often limited by secondary compounds (Silanikove et al., 1994). Tannin is the most common secondary compound in shrub species (Makkar and Becker, 1998).

Goats that browse on shrublands cannot avoid consuming secondary compounds that occur naturally as a part of their defense mechanism against insects and herbivores (Makkar, 2003). Tannins' impacts on animals' health or digestive system are mainly dependent on their structure and concentration in feeds. The diets that have high tannin concentration are decreased feed intake and digestibility (Silanikove et al., 1997a).

The animals have some defense mechanisms avoiding the negative effects of secondary compounds such as the basis of behavior and metabolic pathway such as decreasing the amount of intake, escaping from consumption, consuming mixed with different plant species and producing proline-rich saliva (Shimada, 2006). To avoid the adverse effects of tannin, different techniques are used, such as drying (Ben Salem et al., 1997), alkali treatment (Ben Salem et al., 2005), and polyethylene glycol (PEG) supplementation (Makkar et al., 1995). In general polyethylene glycol (PEG) is used for binding tannin and increases the intake of high tannin-containing shrubs by goats (Silanikove et al., 1997b). Another approach is to supplement animals with different nutrient sources. Supplementary feeding with different feed sources can dilute the adverse effect of tannins (Khan et al., 2009). It's reported that offering supplemental feeds to sheep and goats increased their shrub intake and time to spend browsing (Provenza et al., 2003; Rogosic et al., 2008). Rogosic et al. (2011) reported that supplementary feeding with calcium hydroxide plus barley grain and barley alone enhances the intake of three shrub species. Furthermore *in vitro* gas production method is a useful tool for evaluating the effect of the secondary compound on rumen fermentation (Makkar, 2005). The *in vitro* gas production method allows estimating metabolizable energy (ME), organic matter digestibility (OMD) value (Menke et al., 1979), microbial protein and volatile fatty acids production of shrub species (Blümmel et al., 2003). Shrub and tree leaves contain a certain amount of secondary compounds and using an agent like polyethylene glycol (PEG) in *in vitro* incubations allows for determining the activity of tannins (Ammar et al., 2005). Getachew et al. (2001) reported that adding polyethylene glycol (PEG) in *in vitro* incubations increases short-chain fatty acids and gas production.

This study aims to investigate the influence of harvested stage of *Quercus coccifera L., Phillyrea latifolia L., Ephedra major L. and Spartium junceum L.* shrubs on chemical composition and *in vitro* fermentation characteristics and the effect of polyethylene glycol and concentrate supplementation on fermentation kinetics in *in vitro* incubations.

Material and methods

Ethics Approval

All experimental procedures were approved (2010/11-3) by Animal Care and Use Committee at Çanakkale Onsekiz Mart University.

Study area

The study was conducted at the Technological Agricultural Research Centre (TETAM) of Çanakkale Onsekiz Mart University in Çanakkale.

Shrub samples

Quercus coccifera L., Phillyrea latifolia L., Ephedra major L. and Spartium juncecum L. were the shrub material of this study. Leave samples were harvested from *Quercus coccifera L., Phillyrea latifolia L., Ephedra major L. and Spartium juncecum L.* in March, April, June, July, September and October in the middle of every month (15±3). The shrubs were labeled with plastic plates to obtain samples from the same tree for every sampling period throughout the study. Leaves were harvested from 10 trees for *Quercus coccifera L.* due to the most widespread species in the study area; from 7 trees for the other three species (*Phillyrea latifolia L., Ephedra major L.* and *Spartium juncecum L.*) in the study. All samples were hand-harvested, similar to those consumed by goats were collected.

Leaves samples were dried at room temperature for 10 days on the laboratory bench after the samples were oven-dried at 40^{o}C for 72 h and then ground in a mill to pass through a 1mm screen before. The ground materials were mixed with an equal weight with the sample of the same sampling period for *in vitro* incubations.

Chemical analysis

Dry matter (DM) was determined by drying the samples 105 °C over the night, ash by igniting the samples in a muffle furnace at 550 °C for 4 h and nitrogen (N) content was measured by the Kjeldahl method according to (AOAC, 1990). Crude protein (CP) was calculated by multiplying N x 6.25. The neutral detergent fiber (NDF, Van Soest et al. 1991), acid detergent fiber (ADF) and acid detergent lignin (ADL) analyses used an ANKOM 200 Fiber Analyzer (ANKOM® Technology). NDF was analyzed with sodium sulfite; NDF and ADF are expressed with residual ash in the study. Condensed tannins were determined by using the Butanol-HCL method (Porter et al. 1986) with the modification of Makkar (2003). Total phenols and total tannins in the extracts were estimated using Folin- Ciocalteu reagent using tannic acid as a standard, using polyvinylpolypyrrolidone (PVPP) to separate tannin phenols from non-tannin phenols (Makkar, 2003). Concentrations of all phenolic compounds were expressed in g/kg DM, tannic acid equivalent. The total tannin content was calculated by subtracting the non- tannin phenols from total phenols. All chemical analyses were carried out in two parallels.

***In vitro* gas production**

Rumen fluid was obtained from three canulated non-lactating, non-pregnant Turkish Saanen goats (mean body weight 29.8±1.6 kg) fed twice daily with a diet containing alfalfa hay (60 %) and concentrate (40 %) at an approximately 1.25 times maintenance metabolizable energy (ME) level according to NRC (2007). A sample of rumen content was collected before the morning meal in thermos flasks and taken immediately to the laboratory and samples were mixed in equal volumes and incubations *in vitro* were established according to Menke and Steingass (1988). Three separate incubation sets were run for each shrub species and all samples for each sampling period were put in the same incubation sets. For this purpose 200 mg samples were incubated in 100 ml calibrated glass syringes of each sample in duplicate. The effects of PEG and concentrate feed in *in vitro* gas production was determined by the addition of 40 mg PEG (6000 Sigma Chemical Co. UK) and 20 mg concentrate (maize and soybean meal (60:40, w:w) in duplicate syringes within the same incubation set. The syringes were pre-warmed at 39 °C before the injecting a 30 ml rumen fluid-buffer solution mixture of rumen fluid: buffer solution in a 1:2 ratio was added to each syringe.

The incubations were run with a total of 56 syringes (two syringes of each duplicate sample within each of the six sampling periods and treatments).

Gas production was recorded at 0, 4, 8, 12, 24, 48, 72, and 96 h of incubations. Gas production data were corrected using blanks. Cumulative gas production data were fitted to the exponential equation of Orskov and McDonald (1979), $Y= a+b\ (1\text{-exp-}^{ct})$. Y is presented gas volume (ml) at a time (t), a is the gas produced from the soluble fraction (ml), b the gas produced from an insoluble but fermentable fraction (ml), a+b potential gas production (ml) and the c the rate constant of gas production during incubation (ml h^{-1}), metabolizable energy (ME) and organic matter digestibility (OMD) of the plants was calculating from the gas production according to Menke et al. (1979).

$$\text{ME (MJ/kg DM)} = 2.20 + 0.136\ GP + 0.057\ CP^2 \quad \text{(Formula 1)}$$

$$\text{OMD (\%)} = 14.88 + 0.889\ GP + 0.45\ CP + 0.0651\ A \quad \text{(Formula 2)}$$

Where GP is 24 h gas production (ml/200 mg), CP crude protein content (%), A ash content (%)

Estimating the potential nutritive value of shrub species to meet the needs of a model goat for practical feeding conditions

For this purpose, an adult goat (60 kg body weight) was taken as a model animal. Nutrient requirements (ME and CP) of the model goat were determined according to the NRC (2007). Nutritive value of *Quercus coccifera L.*, *Phillyrea latifolia L.*, *Ephedra major L.* and *Spartium junceum L.* were assessed according to nutrient concentration to meet the requirements of the model goat. The ME and CP results obtained from the spring sampling periods (March and April) were used for calculating the early lactational requirements of the model goat and the results of the summer sampling periods (June and July) were used for calculating the mid-lactational requirements and results obtained from the autumn sampling periods (September and October) were used for calculating the late-lactational requirements. The mean ME or CP concentrations of two sampling periods were used for one season. The comparisons were made by the concentration of the ME and CP requirements calculated based on the daily dry matter intake level for maintenance and different lactation stages reported by NRC (2007) and the ME and CP concentration of shrub species at different sampling periods.

Statistical analysis

The chemical composition of shrub species was analyzed by repeated measurement analysis of variance in a linear model with sampling period as the main factor. Differences between means were determined using the Tukey test. The data of *in vitro* gas production and estimated parameters were analyzed by repeated measurement analysis of variance method using a linear model with sampling period, treatment and sampling period x treatment interactions was the main factor. All data that obtained from the study were analyzed using GLM procedure of SAS (1999).

Results

Chemical composition of shrub species

The chemical composition of the leaves harvested from four shrub species at different sampling periods is presented in Table 1. The CP content of the leaves varied between 49.38 to 97.87 g/kg DM in the study. The sampling periods significantly affected CP content of *Phillyrea latifolia L.*, *Ephedra major L.* and *Spartium junceum L.* leaves (P<0.05). The NDF and ADF content of the shrub species were significantly affected by sampling periods (P<0.05). The ADL contents of *Phillyrea latifolia L.* and *Ephedra major L.* were significantly affected by the sampling periods in the study (P<0.05). The ash contents of *Quercus coccifera L.* and *Spartium junceum L.* were significantly affected by the sampling periods (P<0.05). The ash content of the shrub species ranged from 34.24 to 84.41 g/kg DM.

The CT concentration in *Phillyrea latifolia L.* (P=0.0001) and *Spartium junceum L.* was significantly changed by sampling periods (P<0.0001). TP and TT concentrations were significantly changed in all shrub species according to sampling periods (P<0.05).

Table 2. Least square means (LSM) and standard error of means (SEM) for the chemical composition of shrub species at different sampling periods

Sampling periods	Chemical Composition [1]								
	DM	CP	NDF	ADF	ADL	Ash	CT	TP	TT
Quercus coccifera L.									
March	637.10[b]	49.38	510.84[a]	351.86[bc]	147.21	47.90[b]	19.26	22.24[ab]	19.78[ab]
April	606.90[c]	54.81	468.58[b]	363.99[ab]	171.53	47.35[b]	19.06	22.45[ab]	19.78[ab]
June	627.20[ab]	54.58	518.63[a]	388.98[a]	221.67	34.24[a]	17.09	20.41[b]	18.17[b]
July	594.50[c]	55.49	481.38[b]	362.97[ab]	182.66	48.13[b]	18.66	21.58[ab]	18.98[b]
September	658.30[a]	63.04	447.41[b]	347.02[bc]	180.10	42.69[ab]	18.81	25.50[a]	22.89[a]
October	655.40[a]	63.68	460.09[b]	330.57[c]	192.18	44.99[b]	18.83	21.72[ab]	19.16[ab]
SEM	3.87	3.06	4.14	4.90	14.04	1.55	0.61	0.09	0.69
P	0.0016	0.0921	0.0003	0.002	0.1041	0.0048	0.2813	0.0371	0.0301
Phillyrea latifolia L.									
March	618.05[b]	61.91[ab]	498.74[a]	341.24[a]	159.03[a]	41.86	2.31[bc]	18.69[a]	16.44[a]
April	556.90[c]	58.15[ab]	483.76[a]	341.55[a]	151.35[a]	50.40	2.11[bcd]	19.61[a]	17.72[a]
June	599.00[b]	65.79[ab]	432.10[b]	290.57[b]	94.82[b]	41.90	2.09[bd]	11.95[b]	10.54[b]
July	581.70[b]	61.63[ab]	384.61[cd]	285.22[b]	168.54[a]	42.79	2.73[a]	7.55[c]	5.42[c]
September	691.00[a]	74.00[a]	393.87[c]	288.65[b]	154.64[a]	50.59	2.34[c]	20.43[a]	18.14[a]
October	622.70[a]	54.64[b]	363.08[d]	267.00[b]	147.76[a]	42.66	1.90[d]	13.04[b]	11.51[b]
SEM	3.96	2.81	4.21	4.58	8.62	4.00	0.04	0.47	0.47
P	0.0002	0.0285	<.0001	<.0001	0.0089	0.4366	0.0001	<.0001	<.0001

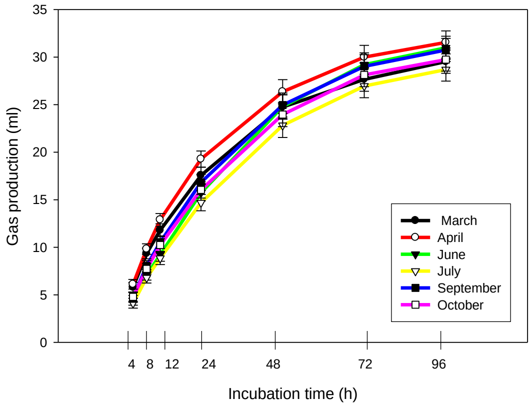
Ephedra major L.									
March	554.10[bc]	72.93[b]	450.91[b]	377.41[a]	222.93[a]	78.73	19.23	27.69 [bc]	23.55 [ab]
April	588.70[ab]	71.49[b]	373.19[c]	290.83[c]	113.90[b]	68.07	19.34	31.35 [a]	27.34 [a]
June	534.20[bc]	81.92[ab]	440.86[b]	344.86[b]	161.43[ab]	66.29	19.03	21.35 [c]	19.12 [c]
July	545.50[c]	81.75[ab]	456.71[b]	295.15[c]	113.55[b]	75.94	19.20	26.66 [b]	22.90 [b]
September	642.80[abc]	79.38[ab]	500.74[a]	351.78[ab]	178.68[ab]	73.16	19.18	28.45 [ab]	24.68 [ab]
October	619.70[a]	89.43[a]	383.95[c]	286.92[c]	143.49[ab]	84.41	19.53	27.30 [b]	23.24 [ab]
SEM	4.04	2.85	7.59	5.10	15.34	4.83	0.08	0.68	0.73
P	0.0361	0.0329	0.0002	<.0001	0.0149	0.2805	0.0630	0.0008	0.0018
Spartium junceum L.									
March	522.70[a]	77.81[b]	362.55[b]	264.21[b]	141.16	34.27[c]	1.55 [cd]	4.04 [c]	3.42 [b]
April	504.90[c]	90.99[ab]	377.48[b]	285.45[b]	161.02	43.28[c]	1.88 [c]	4.97 [b]	4.35 [a]
June	494.75[d]	97.87[a]	372.93[b]	285.45[b]	166.70	59.81[b]	2.94 [b]	3.82 [bd]	3.27 [b]
July	600.50[ab]	83.41[ab]	377.31[b]	290.30[b]	177.51	82.22[a]	1.09 [d]	3.50 [d]	3.00 [b]
September	585.45[b]	79.56[b]	426.29[a]	364.45[a]	151.33	49.31[bc]	7.86 [a]	5.14 [ab]	4.50 [a]
October	590.60[ab]	91.78[ab]	395.16[b]	296.51[b]	143.54	50.05[bc]	2.81 [b]	5.56 [a]	4.82 [a]
SEM	4.12	2.68	6.97	14.63	17.82	2.91	0.11	0.09	0.09
P	0.0010	0.0104	<.0001	0.0005	0.6880	0.0003	<.0001	<.0001	<.0001

[a,b,c,d] Means with different superscripts in the same column are different (P<0.05)
[1]DM, dry matter, g/kg; CP, crude protein, g/kg DM; NDF, neutral detergent fiber, g/kg DM; ADF, acid detergent fiber, g/kg DM; ash, g/kg DM, CT, condensed tannins, g/kg DM; TP, total phenol, g/kg DM; TT, total tannin, g/kg DM

In vitro gas production of shrub species

The cumulative gas productions of shrub species at different sampling periods are shown in Figure 1, 2, 3 and 4 respectively. The cumulative gas production of *Quercus coccifera L.*, *Phillyrea latifolia L.* and *Ephedra major L.* were significantly changed by sampling periods (P<0.05). The highest cumulative gas production was determined in April (31.54; 43.65 ml) while the lowest was determined in July (28.69 and 35.52 ml) for *Quercus coccifera L.* and *Phillyrea latifolia L.* respectively (Figure 1, 2). In comparison, the highest cumulative gas production was determined in June (39.73; 46.92 ml) while the lowest was determined in March (30.34; 40.08 ml) for *Ephedra major L.* and *Spartium junceum L.* respectively (Figure 3,4).

The ME, OMD and incubation parameters are presented in Table 2. The OMD and ME values of shrub species were significantly affected by sampling periods except for *Spartium junceum L.* (P<0.05). The OMD values ranged between 52.52 % to 56.56 % for *Quercus coccifera L.*, 64.85% to 75.15% for Phillyrea *latifolia L.*, 66.96% to 71.10% for *Spartium junceum L.* and 56.59 % to 66.23% for *Ephedra major L*. The “a” value for *Quercus coccifera L.*, *Phillyrea latifolia L.* and *Spratium junceum L.* were significantly affected by sampling periods (P<0.05). The highest “a” value was obtained from *Phillyrea latifolia L.*. Except for *Ephedra major L.* “b” value was significantly affected by sampling periods (P<0.05). The “c” value of *Phillyrea latifolia L.* and *Spratium junceum L.* did not affect by sampling periods. The highest “c” value was determined in *Phillyrea latifolia L.* (mean 0.075 h^{-1}) while the lowest was *Quercus coccifera L.* (mean 0.029 h^{-1}).



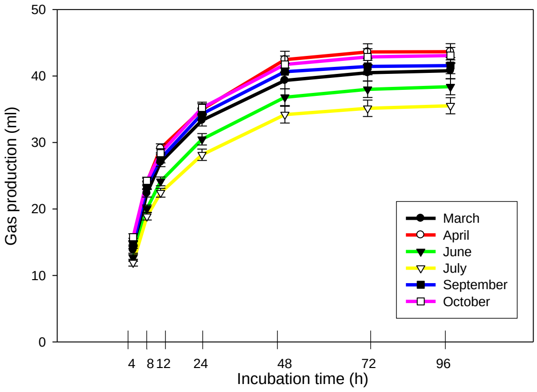
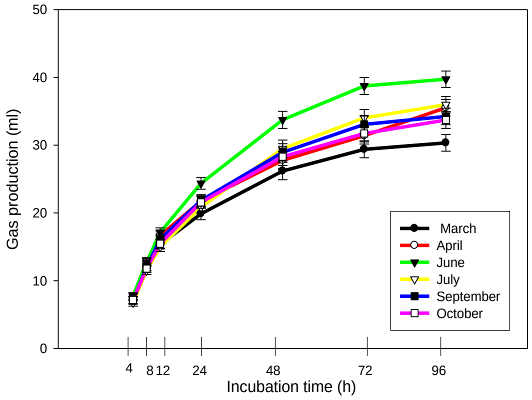


Figure 1. Cumulative gas production of *Quercus coccifera L.* **Figure 2. Cumulative gas production of *Phillyrea latifolia L.***



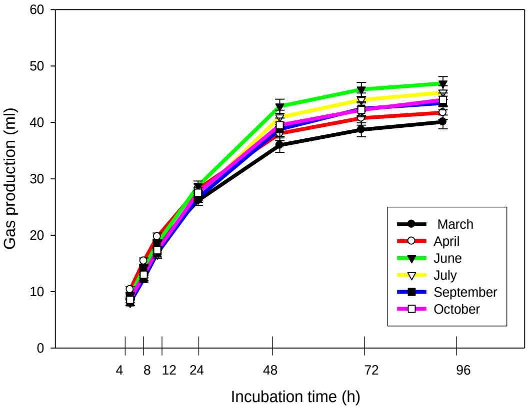


Figure 3. Cumulative gas production of *Ephedra major L.* **Figure 4. Cumulative gas production of *Spartium junceum L.***

Table 2. Least square means (LSM) and standard error of means (SEM) for metabolizable energy (ME), organic matter digestibility (OMD) and incubation parameters

Sampling periods	OMD	ME	Incubation parameters [1] a	b	c
Quercus coccifera L.					
March	52.52 ± 0.67^{b}	8.99 ± 0.12^{b}	1.53 ± 0.14^{b}	31.16 ± 0.63^{abc}	0.025 ± 0.002^{b}
April	55.16 ± 0.67^{ab}	9.41 ± 0.12^{ab}	1.95 ± 0.14^{b}	31.85 ± 0.63^{ab}	0.029 ± 0.002^{ab}
June	54.77 ± 0.67^{ab}	9.41 ± 0.12^{ab}	1.63 ± 0.14^{b}	33.80 ± 0.63^{a}	0.025 ± 0.002^{b}
July	54.10 ± 0.67^{ab}	9.22 ± 0.12^{ab}	2.10 ± 0.14^{b}	30.86 ± 0.63^{bc}	0.027 ± 0.002^{ab}
September	53.94 ± 0.73^{ab}	9.11 ± 0.13^{ab}	3.45 ± 0.14^{a}	28.70 ± 0.63^{c}	0.032 ± 0.002^{ab}
October	56.56 ± 0.67^{a}	9.53 ± 0.12^{a}	3.06 ± 0.14^{a}	30.02 ± 0.63^{bc}	0.034 ± 0.002^{a}
P	0.0023	0.0172	<0.0001	<0.0001	0.0121
Phillyrea latifolia L.					
March	64.85 ± 0.99^{c}	10.65 ± 0.16^{c}	6.16 ± 0.74^{b}	30.47 ± 0.70^{b}	0.072 ± 0.007
April	72.65 ± 0.99^{a}	11.95 ± 0.16^{b}	7.22 ± 0.74^{b}	35.79 ± 0.70^{a}	0.079 ± 0.007

June	68.23±0.99^{bc}	11.22±0.16^{ac}	6.51±0.74^{b}	33.72±0.70^{a}	0.070±0.007
July	74.37±1.32^{a}	12.21±0.16^{b}	10.96±0.74^{a}	33.82±0.70^{a}	0.073±0.007
September	71.69±1.07^{ab}	14.74±0.17^{ab}	7.59±0.74^{b}	35.90±0.70^{a}	0.079±0.007
October	75.15±0.99^{a}	12.32±0.16^{b}	6.46±0.74^{b}	36.58±0.70^{a}	0.074±0.007
P	0.0001	0.0001	0.0003	<.0001	0.8905
Ephedra major L.					
March	61.84±0.71^{b}	10.50±0.12^{c}	3.14±0.46	35.58±1.16^{ab}	0.031±1.16^{b}
April	60.79±0.71^{b}	10.22±0.12^{bc}	3.34±0.46	32.45±1.16^{bc}	0.038±1.16^{ab}
June	66.23±0.71^{a}	11.25±0.12^{a}	3.33±0.46	38.38±1.16^{a}	0.036±1.16^{b}
July	60.11±0.71^{b}	10.09±0.12^{bc}	3.85±0.46	31.60±1.16^{bc}	0.038±1.16^{ab}
September	56.59±0.76^{c}	9.46±0.13^{d}	3.72±0.46	28.09±1.16^{c}	0.037±1.16^{ab}
October	59.49±0.71^{bc}	9.94±0.12^{bd}	3.69±0.46	29.40±1.16^{c}	0.043±1.16^{a}
P	0.0001	0.0001	0.8687	<.0001	0.0013
Spartium junceum L.					
March	71.10±2.40	12.25±0.43	2.75±0.24^{bc}	36.33±1.85	0.034±0.002
April	69.54±2.40	11.93±0.43	2.41±0.24^{c}	38.14±0.24	0.036±0.002
June	73.46±2.40	12.64±0.43	3.47±0.24^{ab}	39.57±0.24	0.036±0.002
July	70.30±2.40	12.05±0.43	2.98±0.24^{bc}	36.46±0.24	0.037±0.002
September	66.96±2.59	11.30±0.47	4.23±0.24^{a}	38.45±0.24	0.038±0.002
October	69.29±2.40	11.70±0.43	4.29±0.24^{a}	35.13±0.24	0.042±0.002
P	0.5847	0.3870	<.0001	0.3707	0.2469

[a,b,c,d] Means with different superscripts in the same column are different (P<0.05)
[1]OMD, %; ME, MJ ME/kg DM; a, gas production from soluble fraction, ml; b, gas production from insoluble fraction, ml; c, gas production rate constant, ml/ h^{-1}

Effects of polyethylene glycol (PEG) and concentrate (CT) supplementation on *in vitro* fermentation characteristics

The cumulative gas production and incubation parameters did not change (P >0.05) by sampling period x treatment interactions in four shrub species in this study. However, the effects of treatments significantly increased the cumulative gas production in *Quercus coccifera L.* and *Ephedra major L.* (P<0.05). The effects of treatments in *in vitro* incubations on cumulative gas production are shown in Figures 5, 6, 7 and 8. A significantly higher cumulative gas production was obtained from PEG and PEG+CT treatments in *Quercus coccifera L.* than in Control (P < 0.05). According to Control, PEG and PEG+CT supplementation increased the gas production in *Quercus coccifera L.* by 11.8% and 10.5%, respectively. The treatments did not affect the cumulative gas production of *Phillyrea latifolia L.* and *Spartium junceum L.* (P>0.05). PEG, PEG+CT, and CT treatments were significantly increased (P<0.05) cumulative gas production in *Ephedra major L.* (Figure 7). There were no differences between the gas production from PEG and PEG+CT treatments in Ephedra major L. (P>0.05). PEG, PEG+CT, and CT treatments were significantly increased (P<0.05) cumulative gas production in *Ephedra major L.* (Figure 7). There were no differences between the gas production from PEG and PEG+CT treatments in *Ephedra major L.* (P>0.05). The supplementation of PEG and PEG+CT increased the gas production of *Ephedra major L.* by 23.8% and 27.5 % respectively compared to Control. Furthermore, according to Control, the CT addition increases the gas production by 10.6 %.

The effects of treatment on OMD, ME and incubation parameters are shown in Table 2. The treatments significantly affected OMD, ME, "a", "b" and "c" values in *Quercus coccifera L.* and *Ephedra major L.* (P < 0.05). The addition of PEG in *in vitro* incubation increased OMD, ME and "b" values in *Quercus coccifera L.* (Table 4). The addition of PEG+CT increased the “a” value in *Quercus coccifera L.* The supplementations were not affected OMD, ME, “b” and “c” values in *Phillyrea latifolia L.* The additives significantly increase the “a” value in *Phillyrea latifolia L.* PEG and PEG+CT addition increase the “a” and “c” values in *Spartium junceum L.* (P<0.05). The OMD and ME were significantly affected by treatments in *Ephedra major L.*

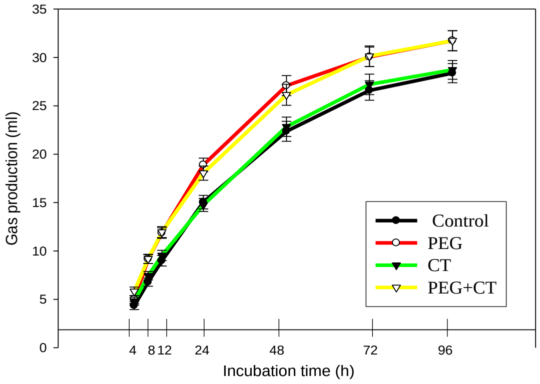


Figure 5. The effects of treatments on cumulative gas production of *Quercus coccifera L.*

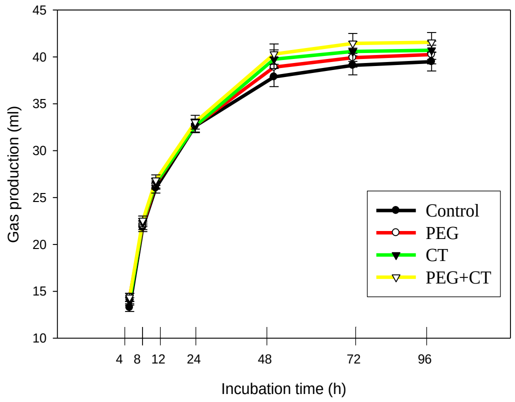


Figure 6. The effects of treatments on cumulative gas production of *Phlyrea latifola L.*

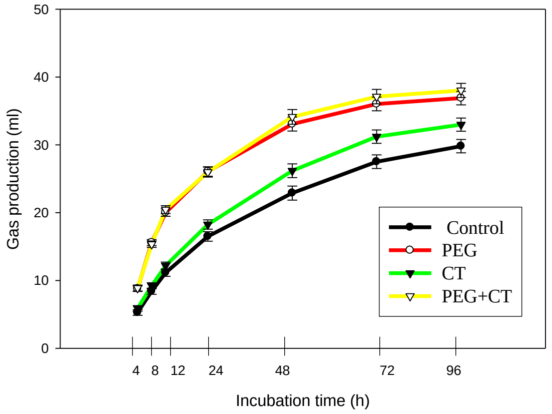


Figure 7. The effects of treatments on cumulative gas production of *Ephedra major L.*

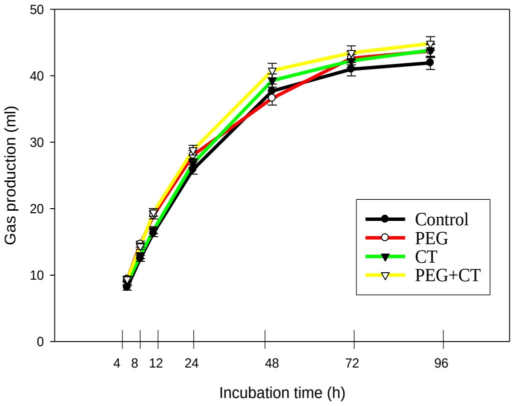


Figure 8. The effects of treatments on cumulative gas production of *Spartium junceum L.*

Estimating the potential nutritive value of shrub species to meet the needs of a model goat

Compare the concentrations of the ME and CP requirements were calculated based on daily dry matter consumption level recommended for maintenance and different lactation stages of adult goats and the nutrient concentration of shrub species shown in Figure 9 and 10. As seen in Figure 9 the ME concentration of shrub species was adequate to meet the maintenance and lactation energy requirements of the model goat. In contrast, the shrub species is insufficient to meet the lactation protein requirements of the model goat but sufficient to meet maintenance requirements.

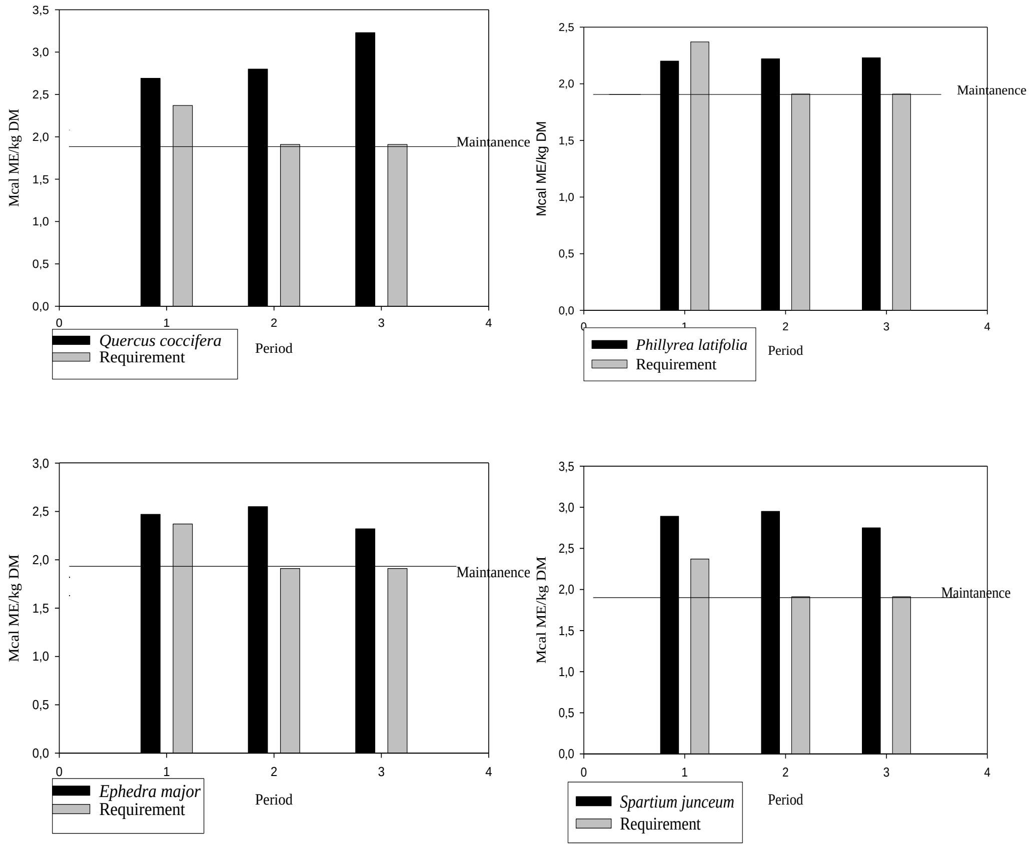
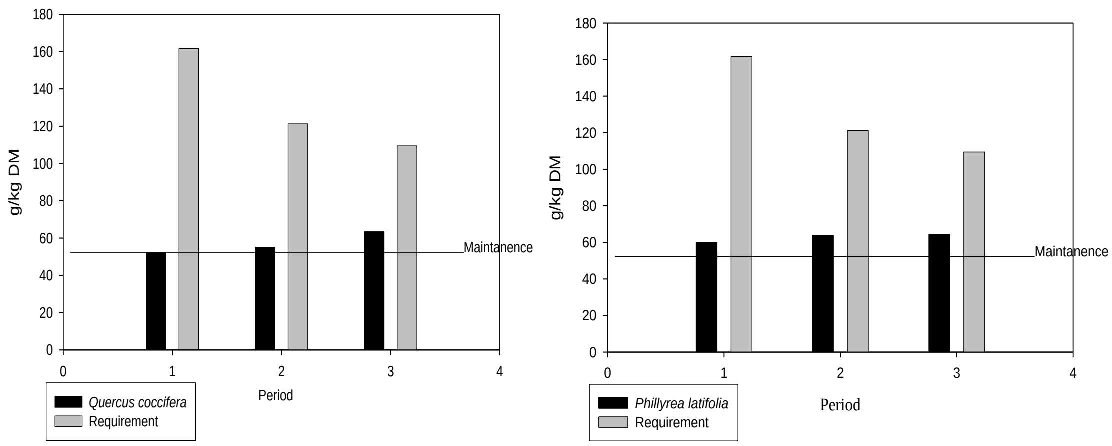


Figure 9 Change in ME concentration of *Quercus coccifera L.*, *Phillyrea latifolia L.*, *Ephedra major L.* and *Spartium junceum L.* by different physiological periods respectively (1: early lactation, 2: mid lactation, 3: late lactation)



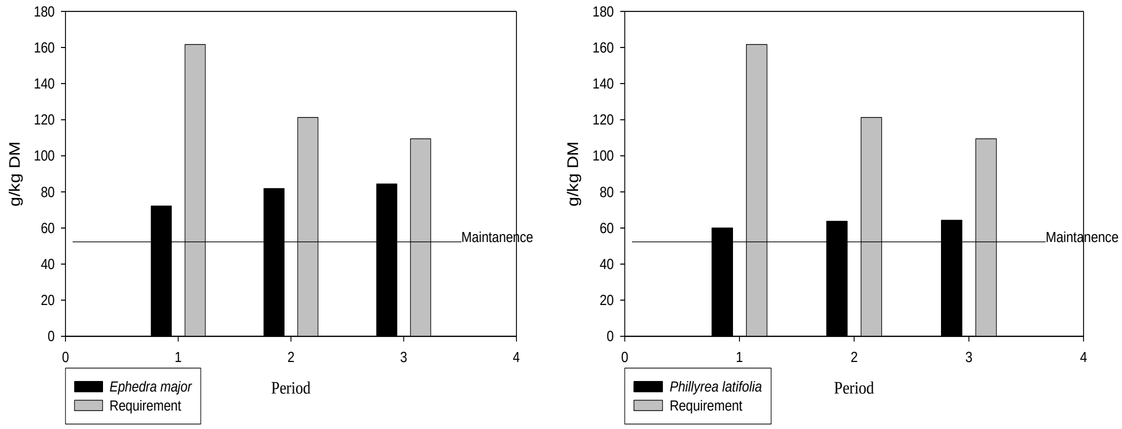


Figure 10 Change in CP concentration of *Quercus coccifera L.*, *Phillyrea latifolia*, *Ephedra major L.* and *Spartium junceum L.* by different physiological periods respectively (1: early lactation, 2: mid lactation, 3: late lactation)

Discussion

The *Quercus coccifera L.*, *Phylrea latifolia L.*, *Ephedra major L.* and *Spartium junceum L.*, shrubs are common in Mediterranean-climate regions (Aydınözü, 2008; Özaslan-Parlak et al., 2011) and are voluntarily consumed by goats (Tölü et al., 2012). The shrub species are generally described as low in protein and high in cell wall constituents (Papanastasis et al., 2008). In this study CP content of *Quercus coccifera L.* (49.38-63.68 g/kg DM) and *Phillyrea latifolia L.* (54.64-74.00 g/kg DM) agreed with the finding of Özaslan-Parlak et al. (2011). The CP content of *Quercus coccifera L.*, *Phillyrea latifolia L.*, *Ephedra major L.* and *Spartium junceum L.* were lower than reported by Tölü et al. (2012). The higher CP content was found in *Spartium junceum L.* and *Ephedra major L.* due to these shrubs belonging to the legume class. It is reported that the CP content of the shrub samples was highest at the beginning of spring due to faster growth and higher cellular activity (Ryan and Bormann, 1982). Afterward, CP content decreased during the growing season, especially in autumn and winter depending on plant maturation (Ammar et al., 2005). The numerically highest CP content was found in the autumn in all shrub samples except for *Spartium junceum L.* in this study, while the differences were not statistically significant between spring and autumn samples in other species ($P > 0.05$). This is probably due to the effects of environmental and soil conditions and a greater proportion of mature leaves in the shrub samples. It has been reported that the cell wall contents in the shrubs change according to the season, especially in the summer months the cell wall components and ash increase and the CP content decrease (Papanastasis et al., 2008). In other words, as the plants develop, the cell wall components such as NDF and ADF are increased (Haddi et al., 2003). Feed NDF content is the best indicator of its intake level and gastrointestinal fullness (Van Soest, 1982). It is known that the quality of forages decreased with the increase in the NDF content. The ADF content of the feed is related to its digestibility. NDF content of *Quercus coccifera L.* and *Phillyrea latifolia L.* were comparable to Kökten et al. (2010) and Özaslan-Parlak et al. (2011), while the ADF content was higher than reported by Kökten et al. (2010). ADL contents of *Quercus coccifera L.* and *Phillyrea latifolia L.* were comparable to Özaslan-Parlak et al. (2011). NDF and ADF content of *Ephedra major L.* and *Spartium junceum L.* were in agreement with the finding of Tölü et al. (2012). In addition, it has been reported in previous studies that the chemical composition of shrub species has wide variation according to the seasons (Castro and Fernandez-Nunez, 2018; Castro et al., 2021), as observed in this study.

Concentrations of secondary compounds varied among shrub species in this study. The concentration of phenolic compounds in shrub species was gave important information about the levels of anti-nutritive compounds, their ability to consume and their nutritive value for animals. It has been reported that the CT concentration of feed that is higher than 50 g/ kg DM has a negative effect on intake and digestibility (Barry and McNabb, 1999). Tannins are the phenolic compounds that suppress the activity of rumen microorganisms and adversely affect animals' performance by decreasing the digestibility of feeds (Min et al., 2003). In addition to the negative effects of tannin, it also reported many positive effects in terms of tannins that can bind to protein and enhance protein by-pass characteristics and have anti-helmintic activity (Ben Salem and Smith, 2008; Lu, 2011). The negative or positive effects of tannins are mainly dependent on chemical composition and concentration in the shrub (Mueller-Harvey, 2006). This study found the highest CT contents in *Quercus coccifera L.* and *Ephedra major L.*, while the lowest contents were found in *Phillyrea latifolia L.* and *Spartium junceum L.* CT content of *Quercus coccifera L.* was a range between 17.09 to 19.26 g/kg DM. Similar results were reported for CT concentration of *Quercus coccifera L.* (Özkan and Şahin, 2006; Ataşoğlu et al., 2010). The mean CT concentration of *Quercus coccifera L.* (18.6 g/kg DM), *Ephedra major L.* (19.3 g/ kg DM) and *Spartium junceum L.* (3.0 g/kg DM) was higher than reported by Tölü et al. (2012) but similar for *Phillyrea latifolia L.* (2.3 g/kg DM). The increasing phenolic contents of plant species may depend on the growth stage and seasonal effect (Frutos et al., 2004) or may be heat stress and the effects of pathogens (Mangan, 1988).

The *in vitro* gas production of shrub species was significantly changed by sampling periods in our study. In April the highest gas production was measured for *Quercus coccifera L.* and *Phillyrea latifolia L.;* the lowest was measured in July for both species. On the contrary, the highest gas production was measured in June for *Ephedra major L.* and *Spartium junceum L.*; the lowest was measured in March for both species. Ammar et al. (2004) reported that *in vitro* gas production of shrubs decreased progressively from spring to autumn due to plants getting maturity and adaptive response to environmental conditions. Mekuriaw et al. (2020) reported that secondary polyphenols were significantly and negatively correlated with gas production, *in vitro* organic matter digestibility and metabolizable energy. The cell wall components especially lignin can be decreased digestibility by inhibiting microbial enzymes from reaching the cell wall (Moore and Jung, 2001). Also, tannins can negatively affect digestibility (Jayanegara et al. 2015) and also can bind to protein or cell wall polysaccharides and inhibit their digestibility (Archana et al., 2010).

The sampling periods had a significant effect on OMD and ME values of *Quercus coccifera L.*, *Phillyrea latifolia L.* and *Ephedra major L.* in the study ($P < 0.05$). Likewise, the incubation parameters were affected by sampling periods ($P < 0.05$). These may be attributed to the fluctuations in nutrient availability and organic matter fermentation and gas production (Osuga et al., 2008). The OMD and ME value of *Quercus coccifera L.* was in agreement with the finding of Ataşoğlu et al. (2010) and Özaslan-Parlak et al. (2011), Eseceli et al. (2020) reported that the ME and OMD value of *Phillyrea latifolia L.* was found 11.38 MJ/kg DM and 75.61% respectively in their study. These results support our findings. The gas production rate (c value) is an important parameter for the description of forage nutritional value and intake level (Khazal et al., 2006). In addition, Blümmel and Becker (1997) reported that the "c" value of forage ranged between 0.032-0.065 ml/ h^{-1}. The "c" value obtained from this study was lower than reported by Blümmel and Becker (1997).

Gas production is an effective method used to determine the effect of the secondary compounds contained in shrubs on rumen fermentation. It has been reported that using PEG in *in vitro* incubations increased the gas production from the feed that contains tannin (Makkar et al., 1995; Getachew et al., 2002; Mekuriaw et al., 2020). The increases in the gas production volume are attributed to increasing the production of volatile fatty acids (VFA) or changing their proportion (Blümmel and Orskov 1993). The PEG is a synthetic polymer that can easily bind to the tannin and increase feed digestion and utilization (Makkar et al., 1995). Increases in gas production with the addition of PEG may be attributed to the increases in the nutrient supply to the rumen microbes (Canbolat et al., 2005). Cumulative gas production of *Quercus coccifera L.* leaves was significantly affected by PEG and PEG+CT treatments in *in vitro* incubations ($P < 0.05$), while the CT treatments did not affect the gas production ($P = 0.9826$). This may be associated with the inadequacy of concentrate feed in eliminating the negative effects of tannin that *Quercus coccifera L.* contained in *in vitro* incubations. The PEG and CT supplementation in *in vitro* incubations increased cumulative gas production of *Ephedra major L.* in this study ($P < 0.05$). On the other hand, the treatments (PEG or CT) did not affect the gas production from *Phillyrea latifolia L.* and *Spartium junceum L.* leaves in *in vitro* incubations ($P > 0.05$). This is probably due to the level and chemical characteristics or activity of condensed tannin because *Quercus coccifera L.* (mean 18.7 g/kg DM) and *Ephedra major L.* (mean 19.3 g/kg DM) shrubs had a higher concentration of CT than the *Phillyrea latifolia L.* and *Spartium junceum L.* (Table 2). Getachew et al. (2002) reported that the use of PEG will increase *in vitro* gas production when the total phenol and tannin content of the samples is higher than 20-40 g/kg DM (g tannic acid equivalent/kg DM). The levels of CT and TP of *Quercus coccifera L.* and *Ephedra major L.* were between the reported values of Getachew et al. (2002).

In the study, one of the treatments was the supplementation of concentrate feed to *in vitro* incubations. The CT treatments did not affect gas production in *Quercus coccifera L.* while having a significant effect in *Ephedra major L.* Although both species have similar CT and TP concentrations, it is concluded that the difference in their structure and chemical composition of condensed tannin or total phenol compounds (Salminen and Caronen 2011). Akbağ (2021) suggested that concentrate, PEG, or their mixture can be used to enhance ruminal fermentation conditions. Rogosic et al. (2011) suggested that supplementation diets with energy feed (barley) or energy feed plus chemical ($Ca(OH)_2$) are an effective method for controlling secondary compound-rich shrubs consumption and their effects on utilization. A supplementation animal with energy sources enhances feed consumption and improves the efficiency of detoxification mechanisms by providing the substrate for eliminating the negative effects of toxins that plants contain (Rogosic et al., 2011).

Comparing the nutrient concentration of shrub species and the maintenance requirement of model goat showed that crude protein seems to be a more important restrictive factor rather than energy. Although *Ephedra major L.* and *Spartium junceum L.* seem to be more unproblematic relative to *Quercus coccifera L.* and *Phillyrea latifolia L.*, it should be noted that comparisons are made at the maintenance levels. To meet the crude protein requirements of the model goat in the early lactation period needs the consume 8.1, 7.0, 5.8 and 5.0 kg DM/day respectively from *Quercus coccifera L.*, *Phillyrea latifolia L.*, *Ephedra major L.*, and *Spartium junceum L.* Based on the theoretical calculations the shrubby vegetation which occur the shrub species deal with in this study needs supplementary feeding with protein concentrates**.** Similarly, Castro et al. (2021) concluded that the shrub species which they used in their study were sufficient to meet the maintenance energy and protein requirements of the model goat, whereas leguminous species were sufficient to only meet the protein requirements in the late gestation period.

Conclusion

The shrub species investigated in this study did not contain secondary compounds that limit intake. The most widespread shrub species is *Quercus coccifera L.* in the Mediterranean shrubby vegetation, while the nutritional value of *Quercus coccifera L.* was lower than other species. *Quercus coccifera L.*, *Phillyrea latifolia L.*, *Ephedra major L.* and *Spartium junceum L.* cannot support CP and ME requirements of high yielding dairy goats. The PEG and PEG+CT supplementation increased the gas production, OMD and ME concentrations in *Quercus coccifera L.* and *Ephedra major L.* The CT supplementation increases the gas production, ME and OMD in *Ephedra major*. The nutritional values of shrubs often have a seasonal variation. Therefore it is important to improve the utilization of shrubs by supplementation and it is necessary to determine the intake level of shrub species to improve supplementation strategies for goats that browse in shrubby vegetation.

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Authors' contributions

I.Y.Y was planning, designing and supervising the experiments. H.I.A. was involved in planning, performing the experiments and writing the manuscript in consultation with I. Y. Y.

Conflict of Interest Statement

The authors declare no conflict of interests

References

Akbağ, H.I., 2021. Potential nutritive value of Anagyris foetida shrubs for goats. Agroforestry Systems, 95 (1):1-10.

AOAC, 1990. Offical Methods of Analysis, 15 th Edition Association of Offical Analytical Chemists, Arlington, VA.

Ammar, H., Lopez, S., Gonzalez, J.S., Ranilla, M.J., 2004. Seasonal variations in the chemical composition and *in vitro* digestibility of some Spanish leguminous shrub species. Anim. Feed Sci. Technol. 115: 327–340.

Ammar, H., Lopez, S., Gonzalez, J.S., 2005. Assessment of the digestibility of some Mediterranean shrubs by *in vitro* techniques. Animal Feed Science and Technology, 119 (3-4): 323-331.

Archana, A., Varsha, B., Jadhav M., Kadam, V.J., 2010. Potential of tannins: A Review. Asian Journal of Plant Sciences, 9 (4): 209-214.

Ataşoğlu, C., Şahin, S., Canbolat, Ö., Baytekin H., 2010. The effect of harvest stage on the potential nutritive value of Kermes Oak (*Quercus coccifera*) leaves. Livestock Research for Rural Development, 22 (2): 182-185.

Aydınözü D., 2008. An investigation on the distribution areas of the maquis formation in Turkey. Kastamonu Education Journal, 16 (1): 207-220.

Barry, T.N., McNabb, W.C., 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. British Journal of Nutrition, 81:163-272.

Ben Salem, H., Smith, T., 2008. Feeding strategies to increase. Small Ruminant Production in Dry Environments, Small Ruminant Research, 77: 174-194.

Ben Salem, H., Nefzaoui, A., Ben Salem, L., Ferchichi, H., Tisserand, J.L., 1997. Intake and digestion in sheep given fresh or air- dried *Acacia cyanophylla* Lindl. foliage. Animal Science, 68: 809-818.

Ben Salem, H., Saghrouni, L., Nefzaoui A., 2005. Attempts to deactivate tannins in fodder shrubs with physical and chemical treatments. Animal Feed Science and Technology, 122: 109-121.

Blummel, M., Becker, K., 1997. The degradability characteristics of 54 roughages and neutral detergent fibre as described by gas production and their relationship to voluntary feed intake. British Journal of Nutrition, 77: 757-768.

Blummel, M., Orskov, E.R., 1993. Comparison of gas production and nylon bag degradability of roughages in predicting feed intake in cattle. Animal Feed Science and Technology, 40: 109-119.

Blummel, M., Karsli, A., Russell, J.R., 2003. Influence of diet on growth yields of rumen microorganisms in vitro and in vivo: influence on growth yield of variable carbon fluxes to fermentation products. British Journal of Nutrition. 90: 625-634.

Canbolat, Ö., Kamalak, A., Özköse, E., Özkan, C.O., Şahin, M., Karabay, P., 2005. Effect of polyethylene glycol on *in vitro* gas production, metabolizable energy and organic matter digestibility of *Quercus cerris* leaves. Lives. Res. Rur. Devel., 17 (4).

Castro, M., Fernandez-Nunez, E., 2018. Feeding value of different plant functional types of oak mediterranean ecosystems. In: 4th European agroforestry conference agroforestry as sustainable land use. Nijmegen, pp. 330–333.

Castro, M., Teixieria, A., Fernandez-Nunez, E., 2021. The nutritive value of different Mediterranean browse species used as animal feeds under oak silvopastoral systems in Northern Portugal. Agroforestry Systems, 95: 269-278.

El Aich, A., 1991. Role of shrubs in ecosystem functions. Options Mediterraneannes Serie A Seminaries Mediterraneens, n. 16: 43-46.

Eseceli, H., Ayaşan, T., Koç, F., Esen, V.K., Esen, S., 2020. Nutrient and mineral concent, and *in vitro* digestibility of Kermes oak (*Quercus coccifera L.*) and Mock Privet (*Phillyrea latifolia L.*). Alinteri J. of Agr. Sci, 35 (2):100-106.

Frutos, P., Hervas, G., Giráldez, F.J., Mantecón, A., 2004. Tannins and ruminant nutrition. Spanish Journal of Agricultural Research, 2: 191-202.

Getachew, G., Makkar, H., Becker, K., 2002. Tropical browses: contents of phenolic compounds, in vitro gas production and stoichiometric relationship between short chain fatty acid and in vitro gas production. The Journal of Agricultural Science, 139, 341-352.

Getachew, G., Makkar, H.P.S., Becker, K., 2001. Method of poliethylene glycol application to tannin-containing browses to improve microbial fermentation and efficiency of microbial protein synthesis from tannin-containing browses. Animal Feed Science and Technology 92: 51-57.

Haddi, M.L., Filacorda, S., Meniai, K., Rollin, F., Susmel, P., 2003. In vitro fermentation kinetics of some halophyte shrubs sampled at three stages of maturity. Animal Feed Science and Technology, 104: 215–225.

Jayanegara, A., Goel, G., Makkar, H., 2015. Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population *in vitro*. Animal Feed Science and Technology, 209: 60-68.

Khan, N. A., Habib, G., Ullah, G., 2009. Chemical composition, rumen degradability, protein utilisation and lactation response to selected tree leaves as substitute of cottonseed cake in the diet of dairy goats. Animal Feed Science and Technology, 154: 160-168.

Khazaal, K., Dentinho, M.T., Riberio, J.M., Orskov, E.R., 1995. Prediction of apparent digestibility and voluntary intake of hays fed to sheep: comparison between using fibre components, *in vitro* digestibility or characteristics of gas production or nylon bag degradation. British Society of Animal Science, 61: 527-538.

Kökten, K., Gürsoy, O., Tukel, T., Hatipoğlu, R., 2010. Yield and nutritive value of Anti-Taurus Mountain rangeland shrubs in Turkey. Journal of Animal and Veterinary Advances, 9 (4): 716-720.

Lu C. D., 2011. Nutritionally related strategies for organic goat production. Small Ruminant Research, 98: 73-82.

Makkar, H.P.S., Becker K., 1998. Adaptation cattle to tannins: role of protein-rich-proteins in Oak fed cattle. Animal Science, 67: 277-281.

Makkar, H.P.S., 2003. Quantification of tannins in tree and shrub foliage a laboratory manual. Kluwer Academic Publishers, Dordrecht/Boston/London/Netherlands.

Makkar, H.P.S., 2005. *In vitro* methods for evaluation of feeds containing phytochemicals. Animal Feed Science and Technology, 123-124: 291-302.

Makkar, H.P.S., Blümmel, M., Becker, K., 1995. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins and their implication in gas production and true digestibility in *in vitro* techniques. British Journal of Nutrition, 73:897–913.

Mangan, J.L., 1988. Nutritional effects of tannins in animal feeds Nutr. Res. Rev.1 pp.209-231

Mekuriaw, S., Tsunekawa, A., Ichinohe, T., Tegegne, F., Haregeweyn, N., Nobuyuki, K., Tassew, A., Mekuriaw, Y., Walie, M., Tsubo, M., 2020. Mitigating the anti-nutritional effect of polyphenols on in vitro digestibility and fermentation characteristics of browse species in north western Ethiopia. Trop. Anim. Health Proc. 52: 1287-1298.

Menke, K.H., Steingass, H., 1988. Estimation of the energetic feed value obtain from the chemical analysis and *in-vitro* gas production using rumen fluid. Animal Research and Development, 28:7–55.

Menke, K., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. The Journal of Agricultural Science, 93: 217-222.

Min, B.R., Barry, T.N., Atwood, G.T., McNabb, W.C., 2003. The effects of condensed tannins on the nutrition and health of ruminants fed fresh temperate forrages: A Review. Animal Feed Science and Technology, 106: 3-19.

Moore, K.J., Jung, H.J.G., 2001. Lignin and fiber digestion. Journal of range management, 54 (4):420-430.

Muller-Harvey I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. Journal of the Food and Agricultere, 86: 2010-2037.

NRC, 2007. Nutrient Requirements of Small Ruminants, National Research Council of the National Academies, Washington, DC.

Ørskov, E., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. The Journal of Agricultural Science, 92, 499-503.

Osuga, I.M., Wambui, C.C., Abdulrazak, S.A., Inhinohe, T., Fujihara, T., 2008 Evaluation of nutritional value and palatability by goats and sheep of selected browse foliages from semiarid area of Kenya.Animal Science Journal 79: 582-589.

Özkan, C.O., Şahin, M., 2006. Comparison of *In situ* dry matter degradation with *in vitro* gas production of Oak leaves supplemented wiht or without polyethylene glycol (PEG). Asian-Aust. J. Anim. Sci. 19 (8): 1120-1126.

Papanastasis, V.P., Yiakoulaki, M.D., Decandia, M., Papanastasis-Dini, O., 2008. Integrating woody species into livestock feeding in the Mediterranean areas of Europe. Animal Feed Science and Technology, 140: 1-17.

Özaslan-Parlak, A., Gökkuş, A., Hakyemez, B.H., Baytekin, H., 2011. Shrub yield and forage quality in Mediterranean shrublands of West Turkey for a period of one year. African Journal of Agricultural Research Vol. 6 (7): 1726-1734.

Papachristou, T.G., Nastis, A.S., Mathur, R., Hutchings, M.R., 2003. Effect of physical and chemical plant defences on herbivory: implications for Mediterranean shrubland management. Basic and Applied Ecology, 4(5): 395-403.

Perevolotsky, A., Landau, S., Kababya, D., Ungar, E.D., 1998. Diet selection in dairy goats grazing woody Mediterranean rangeland. Applied Animal Behaviour Sci. 57: 117–131.

Porter, L.J., Hrstich, L.N., Chan, B.G., 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry, 25: 223-230.

Provenza, F.D., Villalba, J.J., Dziba, L.E., Atwood, S.B., Banner, R.E., 2003. Linking herbivore experience, varied diets and plant biochemical diversity. Small Ruminant Research, 49: 257-274.

Rogosic, J., Estell, R.E., Ivankovic, S., Kezic, J., Razov, J., 2008. Potential mechanisms to increase shrub intake and performance of small ruminants in Mediterranean shrubby ecosystems. Small Ruminant Research, 74: 1-15.
Rogosic, J., Pfister, J.A. Provenza, F.D., Grbesa, D., 2006. Sheep and goat preference for and nutritional value of Mediterranean shrubs. Small Ruminant Research, 64: 169-179.
Rogosic, J., Saric, T., Herceg, N., Zjalic, S., Stanic, S., Scobic, D., 2011. Effects of supplementation with barley and calcium hydroxide on intake of Mediterranean shrubs by goats. Italian Journal of Animal Science, 10:e 23.
Ryan, D.F., Bormann, F.H., 1982. Nutrient resorption in Northern hardwood forests. Bioscience 32: 29-32.
Salminen, J., Karonen, M., 2011. Chemical ecology of tannins and other phenolics: we need a change in approach. Functional Ecology, 25: 325– 338.
Salminen, J.P., Karonen, M., Sinkkonen, J., 2011. Chemical ecology of tannins: Recent developments in tannin chemistry reveal new structures and structure-activity patterns. Chem. Eur. J. 17: 2806–2816.
SAS, 1999. SAS/STAT User's Guide: Version 8. Cary, NC, USA: SAS Institute Inc.
Shimada, T., 2006. Salivary proteins as a defense against dietary tannins. Journal of Chem. Ecol. 32: 1149-1163.
Silanikove, N., Gilboa, N., Nitsan, Z., 1997b. Interactions among tannins, supplementation and polyethylene glycol in goats fed Oak leaves. Animal Science, 64: 479-483.
Silanikove, N., Gilboa, N., Perevootsky, A., Nitsan, Z., 1997a. Effect of foliage-tannins on feeding activity in goats. Options Méditerranéennes: Série A, 34, 43-46.
Silanikove, N., Nitzan, Z., Perevolotsky, A., 1994. Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin-containing leaves (*Ceratonia siliqua*) by sheep. J. Agr. Food Chem. 42: 2844-2847.
Tölü, C., Yurtman, I. Y., Baytekin, H., Savas, T., 2012. Foraging strategies of goats in a pasture of wheat and scrubland. Animal Production Science, 52 (12):1069-1076.
Van Soest PJ., 1982. Nutritional ecology of the ruminant. Cornell University Press, Ithaca, NY, USA.