Ankara Üniv Vet Fak Derg, **67**, 95-100, 2020
DOI: 10.33988/ auvfd.615731

Nutrient content and *in vitro* digestibility of kermes oak (*Quercus coccifera* L.) growing in the provincial borders of Burdur

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Received date: 05.09.2019- Accepted date: 31.10.2019

Abstract: The purpose of this study was to investigate the *in vitro* dry matter digestibility (IVDMD), nutrient content, cell wall components, total and condensed tannin content of kermes oak (*Quercus coccifera* L.) which is the main feed source for goat herds. Kermes oak was exemplified in 3 different stations during a year (12 months). Sampling was made to include leaves, some branches, acorns and young shoots, if any based on season, which were consumed by the goats. The amount of dry matter (DM), crude ash (CA), crude protein (CP), ether extract (EE), crude fibre (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total tannin and condensed tannin content, and IVDMD (by using two-stage technique) were determined on the samples (12 × 3 = 36). Crude protein values were not statistically significant in terms of seasonal averages. On the other hand, it was found that the CF in spring and summer was significantly lower than the CF in winter ($P<0.05$) and the highest EE during the year was in the autumn ($P<0.05$). IVDMD of winter season was significantly lower than summer and autumn. ($P<0.05$). In terms of total tannin content, it was revealed that autumn was significantly lower than winter and spring ($P<0.05$). It was concluded that the nutritional values and IVDMD of kermes oak were low in the winter season.

Keywords: *In vitro*, kermes oak, nutrient composition.

Burdur il sınırları içinde yetişen kermes meşe (*Quercus coccifera* L.) 'nin besin madde içeriği ve *in vitro* sindirilebilirliği

Özet: Bu çalışmanın amacı, keçi sürüleri için ana yem kaynağı olan kermes meşesinin (*Quercus coccifera* L.) *in vitro* kuru madde sindirilebilirliği (IVKMS), besin maddesi içeriği, hücre duvarı bileşenleri, toplam ve kondanse tanen içeriğini belirlemektir. Kermes meşesi yıl boyunca (12 ay) üç farklı istasyondan örneklenmiştir. Örnekleme, keçiler tarafından tüketilen yaprakları, bazı dalları, palamutları ve mevsime göre eğer varsa bazı genç sürgünleri içerecek şekilde yapılmıştır. Örneklere ait kuru madde (KM), ham kül (HK), ham protein (HP), ham yağ (HY), ham selüloz (HS), asit deterjan fiber (ADF), nötral deterjan fiber (NDF), asit deterjan lignin (ADL), toplam ve kondanse tanen içeriği ile IVKMS değerleri (iki aşamalı teknik kullanılarak) belirlenmiştir (12 × 3 = 36). Ham protein değerlerinin, mevsim ortalamaları açısından istatistiksel önem taşımadığı belirlenmiştir. Diğer taraftan ilkbahar ve yaz HS değerinin kışa kıyasla önemli düzeyde düşük ($P<0.05$) ve yıl boyunca en yüksek HY'nin sonbaharda olduğu ($P<0.05$) belirlenmiştir. Kış mevsimi IVKMS, yaz ve sonbahara kıyasla önemli derecede düşük bulunmuştur ($P<0.05$). Toplam tanen içeriği açısından, sonbaharın kış ve ilkbahardan önemli düzeyde düşük olduğu belirlenmiştir ($P<0.05$). Araştırmada kermes meşesinin kış mevsiminde besin değerlerinin ve IVKMS'nin düşük olduğu sonucuna varılmıştır.

Anahtar kelimeler: Besin maddesi, *in vitro*, kermes meşesi.

Introduction

Mediterranean phytogeographical region has a vegetation which includes the evergreen, flaky shrub or shrubs, and kermes oak (*Quercus coccifera* L.) which is the dominant species that constitutes this vegetation (21). Hot and arid summer causes drying of superficially rooted plants; however, deep-rooted plants such as bushes can reach water and remain green (2). It can be considered that it is an important source of roughage for goats, especially if the pasture areas of sufficient quality and quantity are limited. Türkoğlu et al. (33) reported that there is a very strong positive relationship between growth performance of grazing hair goats and the feed mass of the kermes oak. Kamalak et al. (13) reported that oak leaves are rich in CP, CA, NDF, ADF and condensed tannin. However, they are weaker than acorns in terms of *in vitro* organic matter digestibility. Besides the plant parts of oak, the species of oak also affect nutritional properties of plant. For example,

Kökten et al. (16) reported that IVDMD of acorns of *Quercus patreae, Quercus libani, Quercus infectoria, Quercus cercis, Quercus macrolepis, Quercus vulcanica* and *Quercus coccifera* range between 74.67-85.23%. They stated that one of the lowest digestible species is *Quercus coccifera.*

There are some studies, limited in number though, which evaluated the effects of various oak species on the digestibility, feed consumption and fattening performance of goats in the period June - August (19, 34). However, the studies worked on different oak species on dry matter digestibility without concerning seasonal variation (13, 27) and on the nutritive value with ADF and NDF (13, 24). Besides, there are several studies that focused on the nutrient content (10, 13, 15, 26), fatty acid profile (6, 11, 20, 23, 29) and *in vitro* digestibility (10, 25-27, 34) of different species and parts of oak. A study (22) that was encountered in the relevant literature sampled on the main and lateral branch and fresh twigs with leaves of kermes. In the present study, however, samples contained leaves, some branches, acorns and young shoots (if any based on season) all together. Besides, *in vitro* dry matter digestibility of samples were processed by using two-stage digestion method. On the other hand, several fatty acid levels of collected samples were determined. Accordingly, the aim of the study is to evaluate the changes of *in vitro* dry matter digestibility (IVDMD), nutrient content, cell wall components, condensed and extractable tannin content of the samples in order to monitor the changes of nutritional value throughout the year in Burdur Province.

Material and Method

Kermes oaks were sampled from three different bush location within the borders of the Burdur Province. The coordinates of determined stations were 37˚40’33.9”N 30˚19’23.6”E – 37˚40’34.3”N 30˚19’36.0”E and 37˚40’36.4”N 30˚19’22.5”E. Management protocols of the research were made in accordance with approved local ethical committee of Burdur Mehmet Akif Ersoy University (2017-319). Kermes oak samples were collected for 12 months as of January 2017. Winter samples were composed of collected samples from three different station in December 2017, January 2017 and February 2017 (3 × 3 = 9); similarly, the others were composed the samples of each 3 consecutive months of the other seasons of 2017 (9 × 4 = 36 sample). Samples included all parts of plant which was consumed by goats as shown by shepherds. These parts are mixture of leaves, some branches and young shoots, if any based on season. Collected samples were dried in incubator at 65˚C for 48 h (Memmert UE500, F. NR. C593.0011, Memmert GMBH and CO. KG, Germany). Dried kermes oak was milled for analysis in the forage mill (Retsch SK100, 70511012, Retsch GMBH, Germany). Nutrient analysis (DM, CA, CP and EE) of oak samples was performed according to the methods reported in AOAC (3); CF, ADF, NDF and ADL analyses (crude ash free) were determined by using the methods of Crampton and Maynard (7) and Goering and Van Soest (12), respectively. For the analysis of total tannin and condensed tannin contents, oak samples were extracted based on the methods of Terrill et al. (30). Total soluble tannin content was determined by using the method of Lowry et al. (17) and condensed tannin content was determined according to Bate-Smith (5).

Two-stage digestion method was used to determine the changes in dry matter digestibility of oak samples during the seasons. Phosphate-bicarbonate buffer solution and acid-pepsin solution were used at the first and second steps of the method respectively (31). Rumen fluid used in the experiment was obtained from three healthy cow which consumed corn silage and commercial concentrate. The type and amount of fatty acids were determined by gas chromatography (GC/MS, Agilent 5975C, 7890A, Agilent, USA) using the method of Bardakçı and Seçilmiş Canbay (4). For this purpose, the solvent of the oil sample obtained was eliminated by using nitrogen flow and the residue was dissolved in 2 ml of toluene. 5 ml of 1% methanol H_2SO_4 was added and mixed well (8). 5 ml of 2% NaCl solution was added to the mixture which was esterified in water bath at 50ºC for 12 hours. The released fatty acid methyl esters were extracted with hexane. The methyl ester mixture was washed with 5 ml of 2% $KHCO_3$ and dried over anhydrous sodium sulfate. Subsequently, methyl esters were dissolved in 2 ml hexane and analyzed using a gas chromatography. Meteorological records (18) of Burdur province in 2017 were obtained from Burdur Provincial Directorate of Meteorology. According to the climate data, the average temperature was the highest in July (41˚C) and the lowest in January (11.3˚C). The highest precipitation in the year was 60.2 mm in May.

A package program of SPSS was used for statistical analysis (28). Seasonal differences of the investigated parameters were examined through One-Way ANOVA. The significance of mean differences between the seasons was controlled by using Duncan test (14). The results of the investigated parameters were given as marginal means and their standard error of mean. $P<0.05$ was determined for the level of significance (9).

Results

Chemical composition of Kermes oak (*Quercus coccifera* L.) can be seen in Table 1. *In vitro* DM digestibility (IVDMD) and total soluble tannin and condensed tannin levels are presented in Table 2. Fatty acid compositions of samples are provided in Table 3. In

terms of CA values, autumn (3.58%) was found to be significantly lower than summer and winter (P<0.05). On the other hand, there was no statistical difference observed between the mean CA values of spring, summer and winter. It was determined that CP values of samples were ranged from 6.93 to 7.58% throughout the year. The EE content of autumn was determined as the highest (5.54%) when comparing with other seasons (P<0.05). While CF of winter and autumn samples (23.37% and 22.00%) were similar, they were significantly higher than spring and summer averages (P<0.05). In terms of NDF values, the summer is the lowest (47.71%) among the other seasons (P<0.05). Besides, ADF values of summer was higher than the other seasons (P<0.05), while ADF ratio of the spring and autumn were the lowest (P<0.05). There was no statistical significance observed on ADL among the seasons. IVDMD value of winter was lower than summer and autumn (P<0.05). In the autumn, the total soluble tannin content was lower than winter and spring (P<0.05). However, in terms of condensed tannin, there was no difference observed among the seasons. Linoleic acid levels of samples in summer and winter were determined similar, on the other hand, these two season levels were determined higher than spring and autumn (P<0.05).

Table 1. Chemical composition of kermes oak (*Quercus coccifera* L.) samples (% of DM).

Parameters (%)	Winter	Spring	Summer	Autumn	P value
DM	59.10 ± 1.37	57.09 ± 1.22	55.50 ± 1.50	56.77 ± 0.62	0.15
CA	4.37 ± 0.11^{a}	4.03 ± 0.09^{ab}	4.22 ± 0.18^{a}	3.58 ± 0.21^{b}	0.01
CP	7.51 ± 0.26	7.58 ± 0.29	6.93 ± 0.11	7.16 ± 0.35	0.31
EE	3.04 ± 0.28^{c}	3.38 ± 0.27^{c}	4.20 ± 0.20^{b}	5.54 ± 0.33^{a}	0.01
CF	23.37 ± 0.52^{a}	21.00 ± 0.60^{b}	21.58 ± 0.41^{b}	22.00 ± 0.53^{ab}	0.02
NDF	69.28 ± 1.69^{a}	62.44 ± 0.89^{b}	47.71 ± 0.75^{c}	66.58 ± 0.91^{ab}	0.01
ADF	52.23 ± 1.30^{b}	48.37 ± 1.16^{c}	64.13 ± 0.86^{a}	47.62 ± 1.08^{c}	0.01
ADL	34.14 ± 3.71	27.85 ± 1.46	29.48 ± 2.23	27.72 ± 2.07	0.25

n= 9; DM: Dry matter; CA: Crude ash; CP: Crude protein; EE: Ether extract; CF: Crude fibre; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin; ^{a,b,c}: Means in a row with different superscripts are significantly different (P<0.05).

Table 2. *In vitro* dry matter digestibility (%), total and condense tannin (g/kg) contents of Kermes (*Quercus coccifera* L.) (% of DM).

Parameters	Winter	Spring	Summer	Autumn	P value
IVDMD	22.55 ± 0.95^{b}	24.32 ± 0.98^{ab}	27.36 ± 1.58^{a}	27.01 ± 0.70^{a}	0.01
Total tannin	58.59 ± 2.27^{a}	56.91 ± 1.55^{a}	54.86 ± 1.59^{ab}	51.05 ± 1.42^{b}	0.03
Condanse tannin	15.06 ± 2.42	14.33±1.32	12.92±1.27	17.13± 2.03	0.44

n=9; IVDMD: *In vitro* dry matter digestibility; $^{a, b}$: Means in a row with different superscripts are significantly different (P<0.05).

Table 3. Fatty acid composition of Kermes (*Quercus coccifera* L.) oak (% of total methyl esters of fatty acids).

Parameters (%)	Winter	Spring	Summer	Autumn	P value
Myristic acid	5.87 ± 0.12	6.35 ± 0.24	6.40 ± 0.17	6.52 ± 0.18	0.09
Palmitic acid	30.66 ± 0.17	31.46 ± 0.45	31.66 ± 0.46	30.45 ± 0.18	0.05
Palmitoleic acid	2.15 ± 0.05	1.93 ± 0.07	1.87 ± 0.09	1.91 ± 0.11	0.14
Stearic acid	7.69 ± 0.32	8.57 ± 0.37	7.59 ± 0.17	8.54 ± 0.16	0.20
Oleic acid	41.15 ± 0.13	40.65 ± 0.46	40.73 ± 0.38	41.38 ± 0.29	0.39
Linoleic acid	8.57 ± 0.19^{a}	6.82 ± 0.18^{b}	8.07 ± 0.03^{a}	7.07 ± 0.29^{b}	0.01

n= 9; $^{a, b}$: Means in a row with different superscripts are significantly different (P<0.05).

Discussion and Conclusion

In the present study a parallelism was observed between CF and NDF values in terms of 2017 seasons. Although there were seasonal differences, the results of present study were similar to the report of Alatürk et al. (2) who state that NDF and ADF increase with plant maturation. Parlak et al. (22) declared that the level of NDF was about 60% except in April (29.8%), and that the levels of ADF and ADL were about 45% and 19%, respectively during the year. In the present study seasonal ADL levels were determined higher than Parlak et al. (22); on the other hand, generally, the results of ADF and NDF levels were found similar. Moreover, Roukos (25) who worked with the leaves of kermes oak found that the amounts of NDF, ADF and ADL (582, 366 and 132 g/kg dry matter, respectively) was the least in level in August and September when compared to the present study. These differences between the results of investigations may be due to different plant parts with the proportions of the samples. On the other hand, these results confirm that the shrubs consumed by the goats in Burdur, Isparta and its vicinity are rich in cell wall components.

The results of CP levels of kermes oak samples in the present study were similar to Parlak et al. (22) who declared that CP of kermes oak remained approximately 7.5% throughout the year. On the other hand, Roukos (25) found that CP levels of kermes oak leaves were between 14.3% and 14.7% in April and May. It is clear that grazing goats should be fed with additional energy in spring and with additional CP in summer throughout the year in the Mediterranean climate conditions.

It is revealed that CP of sun-drenched twigs and leaves of kermes oak was higher than sunless parts when the plant development was fast (April) (15). Tolunay et al. (32) noted that kermes oak accelerates its spread in March and sprouts in April. They stated that its leaves from the previous season begin to fall in the middle of June. However, we did not find any significant difference among the seasons in the present study. This may be because of the sampling method and the number of the samples. The findings of this study is similar to Alatürk et al. (2) who declared that CP levels of kermes oak are the lowest in summer and the highest in winter and spring. Kamalak et al. (13) found that the CP values of leaves and acorns of kermes oak were 9.17% and 4.23%, respectively. These differences were not seen in the present study because of the sampling method. In conclusion, these studies show that CP levels of different parts or the mixture of all parts of kermes oak are generally low in crude protein.

The EE levels of kermes oak samples in the study were determined as under 5.54% during the year and this result is similar to Alatürk et al. (2) who stated that EE levels of bush species consumed by goats in Isparta Province does not exceed 7.50% seasonally on average.

In vitro dry matter digestibility of Kermes oak was not statistically significant in the spring, summer and autumn while the average of summer and autumn was significantly higher than winter. There was no statistical difference in terms of IVDMD during spring and winter. The fact that the NDF level in the summer was statistically lower compared to the other seasonal averages may have caused the IVDMD to be higher but not statistically significant. However, Roukos (25) revealed that the IVDMD of oak leaves and twigs was significantly lower between August and September compared with the other mounts of year. Parlak et al. (22) declared that dry matter digestibility of kermes oak (Calculated by N and ADF content) was the highest in April (70%), and was between 43.6 to 51.4% in other mounts of year. These results may be due to the use of the difference of methods.

Roukos et al. (26) declared that cell wall digestibility of acorns placed in low altitude and warm climate conditions was higher than placed in high altitude and cold climate conditions. IVDMD of samples in winter was significantly lower than other seasons of the year. This may be caused by high levels of CF and NDF in winter.

Natis and Malechek (19) declared that with the ratio of 46.7%, IVDMD of *Quercus gambelii* was higher than that of the samples in the present study. This difference may be caused by the species of oak. Beside this, Kamalak et al. (13) reported that the leaves of *in vitro* organic matter digestibility of *Quercus coccifera* was 47.05% and this ratio was higher than the present study. It is clear that the difference between the results was caused by the part of plant. On the other hand, similar to the present study, Sidahmed et al. (27) declared that IVDMD of red oak was determined as 29.4%.

It can be concluded that the CP of *Quercus coccifera* is lower than *Quercus persica*, *Quercus infectoria* and *Quercus libani*, and NDF and ADF content of *Quercus coccifera* is similar when these four oak species were compared. Elahi and Rouzbehan (10) stated that the CP levels of *Quercus libani* was 12.3%, ADF levels of *Quercus persica* was 53.2%, and lignin levels of *Quercus infectoria* was 10.3%.

In vitro dry matter digestibility of *Quercus coccifera* did not exceed 27.36% during the 2017 in Burdur province. However, Raju et al. (24) reported that the goats which consume high tannin content have lower IVDMD compared to the goats which consume green grass. Alatürk et al. (2) showed that the highest level of tannins was in the species they examined in sea grape (2.18%) and the lowest in spartium (0.11%). In the present study, condensed tannin content of samples were determined between 12.92 to 17.13 g/kg throughout the year. Kamalak et al. (13) declared that the condensed tannin level of the

leaves of *Quercus coccifera* was 9.22%. Akbağ (1) reported that the condensed tannin content of berm oak in the spring, summer and autumn averages were 19.16; 17.87 and 18.82 g/kg, respectively.

The literature related to the fatty acid composition of oak species focused on the acorns of the plant. When comparing the levels of saturated fatty acids of oak species, the samples of *Quercus coccifera* collected at the present study had more stearic acid levels than the acorns of several oak species. Studies on *Quercus rotundifolia* (29), *Quercus ilex* and *Quercus suber* (6), *Quercus pontica*, *Quercus robur* ssp., *Quercus vulcarinica*, *Quercus pubescens*, *Quercus ithaburensis* ssp. *macrolepis*, *Quercus brantii*, *Quercus libani* and *Quercus trojana* (20), *Quercus robur* L. and *Quercus cerris* L (23), *Quercus suber*, *Quercus rotundifolia* and *Quercus pyrenaica* (11) showed that palmitic acid level was between 12.72-22.8%; stearic acid level was between 0.9-3%; oleic acid level was between 16.9-65.05%; and linoleic acid level was between 13.6-49.1%. It was observed that palmitic and stearic acid ratios of the obtained fatty acids were higher than those of oak samples; oleic acid ratios were similar and linoleic acid ratios were lower. This difference may be due to the fact that the research samples contain all parts of the plant that are consumed by goats.

It was concluded that CF content of kermes oak increased as of summer and reached the highest level in winter. Because of the low IVDMD were determined in winter and the highest NDF content were seen in autumn and winter, it can be said that kermes oak completes its vegetation at the winter in Burdur climate. On the other hand, the plant has the most suitable tannin content for the animals in the autumn months. Because of kermes oak is the main source of feed throughout the year in Burdur, the nutrient requirements of mountain grazing goats are needed to be supported especially in winter and spring months.

Acknowledgements

This study was supported by Burdur Mehmet Akif Ersoy University with the project number of 0470-YL-17. It was summarised from a first author's master's thesis.

Conflict of Interest

The authors declared that there is no conflict of interest.

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