Determination of Chemical Composition, Potential Nutritive Value and Methane Emission of Oak Tree (*Quercus coccifera*) Leaves and Nuts

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Abstract: The current trial was conducted to determine the chemical composition, potential nutritive value and methane production of oak tree leaves and nuts used in small ruminant animals. The chemical composition, metabolisable energy (ME) and *in vitro* organic matter digestibility (IVOMD) of oak tree leaves was significantly different from that of oak nuts (P<0.001). Except for dry matter (DM) and ether extract (EE), the crude ash (CA), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and condensed tannin (CT) contents of oak leaves were significantly higher than those of oak nuts. On the other hand, oil content of leaves and nuts were similar. Although CP, CA and CT contents of leaves were 9.17, 5.58 and 9.22% of DM respectively, NDF and ADF contents of leaves were 45.13 and 41.18% of DM respectively. Gas production, methane production, IVOMD and ME contents of oak nuts were significantly higher than those of leaves (P<0.001). The gas production, methane production, ME and IVOMD of leaves were 27.47, 3.14 ml, 6.65 MJ/kg DM and %47.05 of DM respectively whereas the gas production, methane production, ME and IVOMD of nuts were 66.44 ml, 11.64 ml, 12.45 MJ/kg DM and 77.40% of DM respectively. In conclusion, oak trees from *Quercus coccifera* will provide nut with quite digestible and high ME for small ruminant animals. Oak leaves from *Quercus coccifera* can be used to decrease the methane emission since it has a moderate methane reduction potential. However further investigations are required to determine methane reduction potential of oak leaves *in vivo* animal experiments.
Keywords: *Oak leaves, Oak nuts, nutritive value, condensed tannin, metabolisable energy, in vitro digestibility*

Kermes Meşesi *(Quercus coccifera)* Yaprağı ve Palamudunun Besinsel Kompozisyonu, Potansiyel Besleyici Değeri ve Metan Emisyonu Üzerine Etkisinin Belirlenmesi

Özet: Bu çalışma, küçükbaş hayvan beslemede kullanılan meşe yaprağı ve meşe palamudunun kimyasal kompozisyonunu, besleme değerini ve metan üretimini belirlemek için yapılmıştır. Meşe yaprağının kompozisyonu, metabolik enerji (ME) ve *in vitro* organik madde sindirim derecesi(IVOMSD) meşe palamudundan önemli derecede farklı bulunmuştur(P<0.001). Kuru madde ve yağ içeriği hariç meşe yaprağı HK, HP, NDF, ADF ve KT içeriği meşe palamudundan daha yüksek bulunmuştur. Diğer taraftan yağ içerikleri benzer bulunmuştur. Meşe yaprağının HP, HK ve KT içerikleri kuru madde bazında sırasıyla %9.17, 5.58 ve 9.22 iken, NDF ve ADF içeriği sırasıyla %45.13 ile %41.18 bulunmuştur. Diğer taraftan meşe palamudunun HP içeriği kuru madde bazında %4.23, NDF ve ADF içeriği sırasıyla %28.39 ile %14.49 bulunmuştur. Meşe palamudunun gaz üretimi, metan üretimi, IVOMSD ve ME içeriği meşe yaprağından daha yüksek bulunmuştur(P<0.001). Meşe yaprağının üretilen gaz üretimi, metan üretimi, ME ve IVOMSD sırasıyla 27.47 ml, 3.14 ml, 6.65 MJ/kg KM ve %47.05 of KM bulunmuştur. Diğer taraftan meşe palamudundan üretilen gaz miktarı, metan miktarı, ME ve IVOMSD sırasıyla 66.44 ml, 11.64 ml, 12.45 MJ/kg KM ve %77.40 of KM bulunmuştur. Sonuç olarak kermes meşesi, ruminant hayvanlar için sindirim derecesi ve metabolik enerjisi oldukça yüksek palamut sağlamaktadır. Kermes meşesinin yapraklarının orta seviyede metan üretimini azaltma potansiyeli olduğu için ruminantların rasyonlarında metan üretimini azaltmak için kullanılabilir. Bununla birlikte, geniş çaplı uygulamalara geçilmeden önce meşe yapraklarının metan üretimini azaltma potansiyelleri *in vivo* hayvan denemeleriyle test edilmelidir.
Anahtar Kelimeler: *Meşe yaprağı, meşe palamudu, besleme değeri, kondense tanin, metabolik enerji, in vitro sindirim derecesi*

Introduction

Small ruminant animals consume the oak leaves and nuts to meet their energy, protein and mineral requirements in most parts of world (Ikhimioya et al., 2008; Elahi and Rouzbehan 2008; Kamalak et al., 2010; Kilic et al., 2010; Kaya and Kamalak, 2012). Although there are considerable information about potential nutritive value and chemical composition of oak tree leaves and nuts the information about methane production of oak leaves and nuts is scarce (Kamalak et al., 2005; Kilic et al., 2010; Kaya and Kamalak, 2012). Recently considerable attention has been devoted to decrease methane emission from ruminant animal since due to contribution of methane to global warming and lost of energy consumed by the ruminant animals. It was estimated that approximately 2-12 % of gross energy intake is lost as methane during the fermentation in the rumen , which contributes to climatic change and global warming (Johnson and Johnson, 1995), therefore methane production is called a wasteful process (Getachhew et al., 2005). In vitro gas production technique has been widely used to determine the metabolisable energy and organic matter digestibility (Kamalak et al., 2005; Akçil and Denek, 2014). The current trial was conducted to determine the chemical composition, potential nutritive value and methane production of oak tree leaves and nuts used in small ruminant animals.

Materials and Methods

Preparation of samples of oak leaves and nuts for analysis

Leaves and nuts collected from ten different oak trees (*Quercus coccifera*) in Kahramanmaras, Turkey were analyzed by oven drying at 105 ^{o}C 24 hours. Dried leaves and nuts were milled to pass through 1 mm screen for subsequent analysis. Crude ash contents of oak leaves and nuts were determined by igniting the in muffle furnace at 525 ^{o}C for 8 h (AOAC 1990). Nitrogen (N) contents of oak leaves and nuts were measured by the Kjeldahl method (AOAC 1990). Crude protein contents of oak leaves and nuts were calculated as N X 6.25. Neutral detergent fiber (NDF) contents of oak leaves and nuts were analyzed according to the method described by Van Soest and Wine (1967) and ADF contents of oak leaves and nuts were analyzed according to the method described by Van Soest (1963). Condensed tannin contents of oak leaves and nuts were estimated by butanol-HCl method as suggested by Makkar et al. (1995). All chemical analyses were carried out in triplicate.

Measurements of gas and methane production

Gas and methane production of oak leaves and nuts were determined using the method described by Menke et al. (1979). The rumen fluid was obtained from three fistulated sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). Approximately 200 mg oak leaves and nuts samples were incubated in triplicate into calibrated glass syringes of 100 ml in a water bath at 39 ^{o}C. Total gas productions were corrected for blank incubation. Methane production (%) of total gas produced at 24 h fermentation of oak nuts were measured using an infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goel et al., 2008). Using the plastics syringe, approximately 30-40 ml gas samples were transferred into inlet of the infrared methane analyzer. The infrared methane analyzer displays methane as percent (%) of total gas. Methane production (ml) was calculated as follows.

Methane production (mL) = Total gas production (mL) X Percentage of methane (%)

Determination of metabolisable energy and organic matter digestibility oak leaves and nuts

Metabolisable energy (ME, MJ/kg DM) and IVOMD of oak leaves and nut samples were estimated using equation suggested by Menke and Steingass (1988) as follows:

ME (MJ/kg DM) = 0.72 + 0.1559GP + 0.068CP +0.249EE
IVOMD (%) = 14.88 + 0.8893GP + 0.448CP +0.651CA
Where
GP = 24 h net gas production (ml/200 mg)
CP = Crude protein
EE = Ether extract (%)
CA = Ash content (%)

Statistical Analysis

The differences between oak leaves and nuts composition, gas production, methane production, ME and IVOMD were tested by using t test.

Results

The chemical compositions of oak leaves and nuts are given in Table 1. The chemical composition of oak tree leaves was significantly different from that of oak nuts (P<0.001). Except for DM and EE, the CA, CP, NDF, ADF and CT contents of oak leaves were significantly higher than those of oak nuts. On the other hand, oil content of leaves and nuts were similar. Although CP, CA and CT contents of oak leaves were 9.17, 5.58 and 9.22% of DM respectively, NDF and ADF contents of oak leaves were 45.13% and 41.18% of DM respectively. Gas production, methane production, IVOMD and ME of oak leaves and nuts are given in Table 2. Gas production, methane production, IVOMD and ME contents of oak nuts were significantly higher than those of oak leaves (P<0.001). The gas production, methane production, ME and IVOMD of oak leaves were 27.47, 3.14 ml, 6.65 MJ/kg DM and %47.05 of DM respectively whereas the gas production, methane production, ME and IVOMD of oak nuts were 66.44 ml, 11.64 ml, and 12.45 MJ/kg DM and 77.40% of DM respectively.

Table 1. Chemical composition of oak leaves and nuts

	Oak trees			
Composition (%)	**Leaves**	**Nuts**	**SEM**	**P**
DM	52.26 [b]	64.78 [a]	0.167	<0.001
CA	5.58 [a]	2.36 [b]	0.244	<0.001
CP	9.17 [a]	4.23 [b]	0.123	<0.001
NDF	45.13 [a]	28.39 [b]	0.080	<0.001
ADF	41.88 [a]	14.49 [b]	0.487	<0.001
EE	4.51	4.50	0.039	0.782
CT	9.22 [a]	3.68 [b]	0.310	<0.001

[a b] Row means with common superscripts do not differ (P>0.05); SEM: Standard error mean, DM : Dry matter %, CP : Crude protein %, CA: Crude ash %, NDF : Neutral detergent fiber%, ADF : Acid detergent fiber%, EE : Ether extract (%) CT: Condensed tannin% , Chemical composition of leaves and nuts were expressed as % of DM

Table 2. Gas production, methane production, metabolisable energy, organic matter digestibility of oak tree leaves and nuts

	Oak trees			
Parameters	**Leaves**	**Nuts**	**SEM**	**P**
TG	27.47 [b]	66.44 [a]	0.771	<0.001
CH_4	3.14 [b]	11.64 [a]	0.229	<0.001
CH_4 (%)	11.44 [b]	17.52 [a]	0.292	<0.001
ME	6.65 [b]	12.45 [a]	0.118	<0.001
IVOMD	47.05 [b]	77.40 [a]	0.684	<0.001

[a b] Row means with common superscripts do not differ (P>0.05); SEM : Standard error mean, TG: total gas produced at 24 h incubation (ml/200 mg DM), CH_4 : Methane produced at 24 h incubation (ml/200 mg DM), ME : Metabolically energy (MJ /Kg DM); IVOMD : In vitro organic matter digestibility % of DM.

Discussion and Conclusion

There are significant differences between oak leaves and nuts in terms of chemical composition (P<0.001). Oak leaves are very rich in CP, CA, NDF, ADF and CT when compared with oak nuts. It is desirable that oak leaves are rich in terms of CP but it is not desirable that oak leaves are rich in terms of NDF, ADF and CT. It is well known that NDF, ADF and CT are negatively associated with ME and IVOMD of feedstuffs (Kamalak et al., 2011; Kamalak and Canbolat, 2010). It was reported that CT in feedstuffs have different effects on digestibility and animal performance depending on the amount of tannin. Low level of condensed tannin (2-3%) has a beneficial effect on degradation of protein since low level of condensed tannin prevents the protein from extensive degradation of protein in the rumen (Barry, 1987). On the other hand high level of condensed tannin has negative effect on the digestion of protein since high level of condensed tannin reduces the digestion of protein (Kumar and Singh, 1984). Therefore oak leaves may reduce the digestion of protein in ration due to high level of condensed tannin. However oak nut in ration may reduce the degradation of protein in the rumen due to low level condensed tannin.

As can be seen from Table 2, oak leaves fermentations resulted in low methane production when compared with oak nuts. Low level methane production is associated with high level CT in oak leaves. This result is consistent with findings of Woodward et al. (2004), Carulla et al. (2005) and Tan et al. (2011) who showed that condensed tannin in diets significantly reduced the methane emission.

Lopez et al. (2010) suggested that methane content of total gas produced during fermentation can be used to determine the methane reduction potential of any feedstuffs and the feedstuffs can be classified in three groups, low potential (% methane in gas between >11% and ≤14%), moderate potential (% methane in gas between >6% and <11%), high potential (% methane in gas between >0% and <6%). Therefore it seems to be likely that oak tree leaves studied in the current study had methane reduction potential since the percentage of methane fall into second group.

The reason why oak leaves had lower gas production than that of oak nuts is the higher NDF, ADF and CT contents of oak leaves. The amount of gas produced during the fermentation depends on the amount of fermentable substrate for microorganisms (Blümmel and Orskov, 1993).

Therefore estimated ME and IVOMD of values of oak leaves were significantly lower than those of oak nuts, even if the oak leaves has higher CP content that that of oak nut.

The chemical composition of oak tree leaves was considerably different from that indicated by Kamalak et al. (2005). The differences in chemical composition of oak leaves between two studies possibly associated with growing conditions and maturity of leaves obtained. The chemical composition of oak tree nuts was considerably different from that indicated by Kaya and Kamalak (2012). The differences in chemical composition of oak nuts between two studies are possibly associated with growing conditions and maturity of oak nuts (Baubaker et al., 2007; Maujahed et al., 2005). The ME and IVOMD digestibility of oak nuts were considerably higher than those indicated by Kaya and Kamalak (2012). The differences in ME and IVOMD of oak nuts between two studies are possibly associated with differences in chemical composition of oak nuts.

In conclusion, oak trees from *Quercus coccifera* will provide nut with quite digestible and high metabolisable energy for small ruminant animals. However oak leaves from *Quercus coccifera* can be used to decrease the methane emission since it has a moderate methane reduction potential. However further investiga-tions are required to determine methane reduction potential of oak leaves in vivo animal experiments.

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