

Determination of nutritive value and anti-methanogenic potential of mistletoe leaves (*Viscum album*) grown on different host

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Abstract

The purpose of the current study was to evaluate effect of host on chemical composition, *in vitro* gas production, methane production, metabolisable energy (ME) and organic matter digestibility (OMD). The host had a significant effect on the chemical composition, *in vitro* gas production, methane production, ME and OMD of mistletoe leaves. Crude ash (CA) and crude protein (CP) contents of mistletoe leaves ranged from 5.33 to 8.93 % and 8.53 to 12.16% respectively. The CA and CP contents of leaves of mistletoe grown on *Prunus armeniaca* were significantly higher than the others. The host had no significant effect on the neutral detergent fiber (NDF) contents whereas the host had significant effect on acid detergent fiber (ADF) contents of leaves of mistletoe. NDF and ADF contents of leaves of mistletoe ranged from 32.50 to 34.80% and 19.43 to 21.56 % respectively. Gas and methane production of mistletoe leaves ranged from 39.53 to 44.50 ml, 6.26 to 7.06 ml. Gas production of mistletoe leaves grown on *Prunus domestica* was significantly higher than those of mistletoe leaves grown on *Prunus armeniaca* and *Pirus communis*. ME and OMD of mistletoe leaves ranged from 8.26 to 9.20 MJ (kg /DM) and 51.16 to 55.30 % respectively. ME and OMD of mistletoe leaves grown on *Prunus domestica* was significantly higher than those for *Prunus armeniaca* and *Pirus communis*. Leaves of mistletoe can be used during the food shortage to meet the forage requirement of ruminant animals. However, leaves of mistletoe grown on *Prunus domestica* and *Prunus dulcis* should be supplemented with protein sources to prevent the possible protein deficiencies in mistletoe leave consuming ruminants. Further investigations are required to determine the effect mistletoe leaves on feed intake of ruminant animals.

Key words: Mistletoe leaves, chemical composition, digestibility, gas production, methane production

Introduction

In the most parts of world ruminant animal may face feed shortages during winter when there is available pasture for grazing. During the feed shortages alternative feedstuffs such as mistletoe plant which is semi parasite plant have been used to meet nutrient requirement of ruminant (Umucular et al., 2007, Ramantsi et al., 2019, Ture et al., 2010, Hejcman et al., 2014).

However, the lack of information on the nutritive value of alternative plants is one of the main limiting factors to use in ruminant animals diets. Therefore, more information about

the nutritive value of mistletoe plant for ruminant is required to make sound decisions.

It is well known that fermentation of carbohydrate in rumen not only results in synthesis of valuable end products such as volatile fatty acids and microbial protein but also emission of undesirable products such as CH₄ which are one of the greenhouse gases. The production of CH₄ also results in an energy loss during the fermentation (Johnson and Johnson 1995).

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Although previous studies were conducted mainly on the chemical composition, gas, metabolisable energy and organic matter digestibility of mistletoe plant for ruminant, there is no information available on anti-methanogenic potential of mistletoe plant. Recently chemical analysis along with *in vitro* gas production technique has been used to evaluate the potential nutritive value and anti-methanogenic potential of previously less investigated plant species (Navarro-Villa et al., 2011, Banik et al., 2013, Uslu et al., 2018). The purpose of the current study was to evaluate effect of host on chemical composition, *in vitro* gas production, methane

production, metabolisable energy (ME) and organic matter digestibility (OMD).

Material and Methods

Harvest of mistletoe leaves grown in different host

Mistletoe plants were harvested from different host such as *Prunus domestica*, *Prunus armeniaca*, *Prunus dulcis* and *Pirus communis* in Yozgat, in 2019 and dried in shade. The pictures are given in Figure 1. After drying, samples of mistletoe plants were ground to pass through 1 mm sieve size and stored in nylon bags for subsequent chemical analysis and *in vitro* gas production.

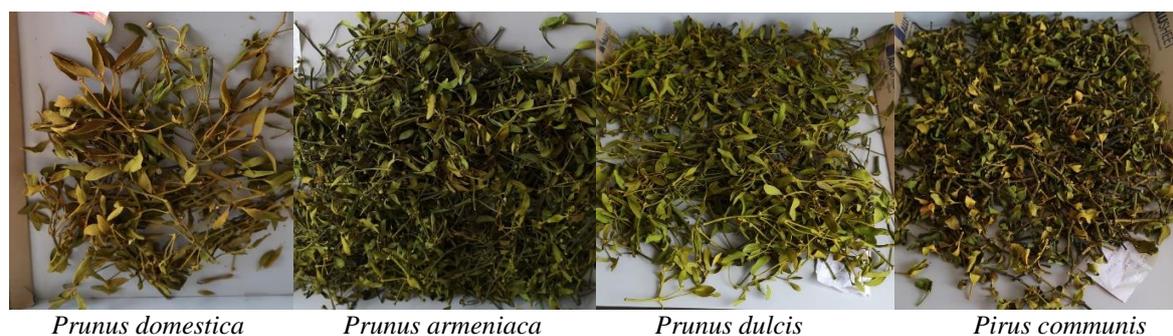


Figure 1. Pictures of host's mistletoe leaves

Chemical analysis of mistletoe leaves

Dry matter (DM), crude ash (CA), crude protein (CP) and ether extract (EE) of mistletoe leaves were determined by the method of AOAC (1990). NDF and ADF contents of mistletoe leaves were determined by the method of Van Soest (1991).

Determination of gas and methane production of mistletoe leaves

Gas (GP) and methane production (MP) of mistletoe leaves were determined using the method suggested by Menke et al., (1979). The rumen fluid was obtained from two fistulated Awassi sheeps fed with alfalfa hay (800g) and barley (400g) and filtered with four layered cheesecloth under flushing with CO₂.

Then 30 ml of the buffered rumen fluid (1:2 V/V) was added into glass syringes containing 200 mg of mistletoe leaves samples and transferred in water bath set at 39 °C for 24 h incubation. All incubation was carried out in quadruplicate. The gas and methane production of mistletoe leaves were measured after 24 h incubation. The percentages of methane of total gas production of mistletoe leaves samples were determined using infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goel et al., 2008). The methane productions of mistletoe leaf samples as mL were calculated as follows:

$$\text{CH}_4 \text{ production (ml)} = \text{Total gas production (ml)} \times \text{Percentage of CH}_4 (\%)$$

Metabolisable energy and organic matter digestibility of mistletoe leaves samples were estimated with equations suggested by Menke and Steingass (1988).

$$\text{ME (MJ/kg DM)} = 2.2 + 0.136\text{GP} + 0.057\text{CP} + 0.0028859\text{EE}^2$$

$$\text{OMD (\%)} = 14.88 + 0.8893\text{GP} + 0.0448\text{CP} + 0.0651\text{CA}$$

GP: gas production of 200 mg sample at 24 h incubation (ml)

CP: Crude protein (%)

EE: Ether extract (%)

CA: Crude ash (%)

The current experiment was carried out with the permission of the Animal Experimentation Ethics Committee of University of Kahramanmaraş Sutcu Imam, Faculty of Agriculture (Protocol No: 2020/02-04).

Statistical Analyses

One-way analysis of variance (ANOVA) was used to determine the effect of host on chemical composition, *in vitro* gas production, methane production, ME and OMD of mistletoe leaves. Differences ($P < 0.05$) among the mean of mistletoe leaves were determined with Tukey's multiple range tests

Results and Discussions

Effect of host on the chemical composition of mistletoe leaves

The effect of host on the chemical composition of mistletoe leaves are given in Table 1. The host had a significant effect on the chemical composition of mistletoe leaves.

The CA and CP contents of mistletoe leaves ranged from 5.33 to 8.93 % and 8.53 to 12.16 % respectively. The CA and CP contents of leaves of mistletoe grown on *Prunus armeniaca* were significantly higher than the others. CP contents of mistletoe leaves obtained in the current experiment were considerable higher than those obtained by Umucular et al., (2007) and Saleh et al., (2015). On the other hand, CP contents of mistletoe leaves obtained in the current experiment were similar to those reported by Hejcman et al., (2014).

CP is very important nutrients for ruminant animal. The feedstuffs should contain at least 10 % of CP to meet the maintenance requirement of ruminant animals. As can be seen from Table 1 the CP contents of leaves of mistletoe grown on *Prunus domestica* and *Prunus dulcis* were lower than the level required for maintenance of ruminant animals.

Therefore, leaves of mistletoe grown on *Prunus domestica* and *Prunus dulcis* should be supplemented with protein

sources to prevent the possible protein deficiencies in mistletoe leave consuming ruminants.

Table 1. Effect of host on chemical composition of mistletoe leaves

Host	DM	CA	CP	EE	NDF	ADF
<i>Prunus domestica</i>	96.16	6.73b	8.53c	12.63a	32.50	19.43b
<i>Prunus armeniaca</i>	95.96	8.93a	12.16a	7.56c	34.80	21.56a
<i>Prunus dulcis</i>	96.00	6.73b	9.40bc	11.76b	34.73	20.13ab
<i>Pirus communis</i>	96.23	5.33c	10.03b	3.86d	33.73	19.63b
SEM	0.129	0.124	0.447	0.239	1.162	0.512
P	0.194	<0.001	<0.001	<0.001	0.240	0.012

^{a b c} Column means with common superscripts do not differ ($P>0.05$), SEM: standard error mean. DM: Dry matter (%), CA: Crude Ash (% of DM), CP: Crude protein (% of DM), EE: Ether extract (% of DM), NDF: Neutral detergent fiber (% of DM), ADF: Acid detergent fiber (% of DM)

Ether extracts of leaves of mistletoe ranged from 3.86 to 12.63 %. EE of leaves of mistletoe grown on *Prunus domestica* was significantly higher than the others. Although EE of mistletoe leaves on *Prunus domestica* and *Prunus dulcis* were significantly higher than those reported by Umucular et al., (2007), EE of mistletoe leaves on *Prunus armeniaca* was comparable to those reported by Umucular et al., (2007). On the other hand EE of mistletoe leaves on *Pirus communis* was significantly lower than those reported by Umucular et al., (2007).

The host had no significant effect on the NDF content whereas the host had significant effect on ADF contents of leaves of mistletoe. NDF and ADF contents of leaves of mistletoe ranged from 32.50 to 34.80 % and 19.43 to 21.56 % respectively. NDF and ADF contents of leaves of mistletoe were consistent with findings of Umucular et al., (2007) who

reported that NDF and ADF content ranged from 29.1 to 33.0 % and 17.1 to 20.2 % respectively.

The differences among the studies in terms of chemical compositions of leaves of mistletoe are possible associated with harvest stage and host on which mistletoe is grown. Umucular et al., (2007) clearly indicated that host and harvesting stage had a significant effect on the chemical composition of leaves of mistletoe.

Effect of host on gas, methane, metabolisable energy and organic matter digestibility of mistletoe leaves

Effects of host on gas, methane, metabolisable energy and organic matter digestibility of mistletoe leaves were shown in Table 2. Host had a significant effect on gas, methane, ME and OMD of mistletoe leaves.

Table 2. Effect of species on gas, methane, metabolisable energy and organic matter digestibility of mistletoe leaves

Host	Gas (ml)	CH ₄ (ml)	CH ₄ (%)	ME	OMD
<i>Prunus domestica</i>	44.50a	7.00a	15.66c	9.20a	55.30a
<i>Prunus armeniaca</i>	39.53b	6.50b	16.50b	8.43b	51.16b
<i>Prunus dulcis</i>	40.93ab	6.26b	15.36c	8.70ab	52.13ab
<i>Pirus communis</i>	40.10b	7.06a	17.60a	8.26b	51.36b
SEM	1.129	0.145	0.266	0.161	1.001
P	0.09	0.001	0.000	0.002	0.011

^{a b c} Column means with common superscripts do not differ ($P>0.05$), SEM: standard error mean. GP: Gas production (ml), CH₄: Methane emission (ml), ME: Metabolisable energy (MJ/kg DM), OMD: Organic matter digestibility (%)

Gas and methane production of mistletoe leaves ranged from 39.53 to 44.50 ml, 6.26 to 7.06 ml. Gas production of mistletoe leaves grown on *Prunus domestica* was significantly higher than those of mistletoe leaves grown on *Prunus armeniaca* and *Pirus communis*. Methane production of mistletoe leaves grown on *Prunus domestica* was significantly higher than the other mistletoe leaves. The percentage of methane in total gas production of mistletoe leaves grown on *Pirus communis* was significantly higher than the others.

The gas production of mistletoe leaves studied in the current experiment was considerably lower than those reported by Umucular et al., (2007) who found that gas production at 24 h incubation ranged from 67.7 to 71.1 ml. The differences in gas production between two experiments are possibly associated with differences in mistletoe leaves studied.

Lopez et al., (2010) reported that the percentage of methane of feedstuffs should be lower than 14 % to have an anti-

methanogenic potential. The percentage of methane of all mistletoe leaves higher than 14 %. Therefore, mistletoe leaves studied in the current experiment had no anti-methanogenic potential

ME and OMD of mistletoe leaves ranged from 8.26 to 9.20 MJ (kg /DM) and 51.16 to 55.30 % respectively. Metabolisable energy and OMD of mistletoe leaves grown on *Prunus domestica* was significantly higher than those for *Prunus armeniaca* and *Pirus communis*. However, ME and OMD of mistletoe leaves studied in the current experiment were comparable with those reported by Umucular et al., (2007) who found that ME and OMD ranged from 7.8 to 8.3 MJ (kg /DM) and 52 to 56 %.

Conclusions

The host had a significant effect on the chemical composition, *in vitro* gas production, methane production, ME and OMD of mistletoe leaves. Leaves of mistletoe can be

used during the food shortage to meet the forage requirement of ruminant animals. However, leaves of mistletoe grown on *Prunus domestica* and *Prunus dulcis* should be supplemented with protein sources to prevent the possible protein deficiencies in mistletoe leaf consuming ruminants. Further investigations are required to determine the effect mistletoe leaves on feed intake of ruminant animals.

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