



## Determination of the Antimethanogenic Properties of Sumac Leaves (*Rhus coriaria L.*) Substitution at Different Ratios Instead of Corn Silage in Sheep Rations by *in Vitro* Gas Production Method

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### ARTICLE INFO

### ABSTRACT

#### Research Article

Received : 26/11/2021

Accepted : 01/02/2022

#### Keywords:

Sumac leaves  
Gas production  
*In vitro*  
Methane  
Feed value

This study was carried out to determine the effects of different doses (10, 20 and 30%) of sumac shrub leaf substitution instead of corn silage in sheep rations on *in vitro* gas and methane production, metabolic energy (ME), net energy lactation (NEL) and organic matter digestion degree. Sheep ration consisting of corn silage (20%), alfalfa straw (22.5%), dry meadow grass (20%), and commercial feed (37.5%) constituted the control group. The experimental groups were formed by substituting 10 (S1), 20 (S2) and 30 (S3) percent sumac shrub leaves for corn silage in the control (C) group formed the experimental groups. The effect of sumac shrub leaf substitution on *in vitro* gas and methane production, metabolic energy, net energy lactation, and organic matter digestion degree was found to be significant. The 24-hour *in vitro* gas production values of rations ranged between 43.11- 46.77 ml/200 mg DM, methane production values 6.8-7.48 ml, metabolic energy values 8.91-9.41 MJ/kg DM, net energy lactation, 5.59-5.95 MJ/kg DM and organic matter digestion degree values found between 64.25 and 67.61%. As a result, it was determined that increasing doses of sumac shrub leaf substitute reduced gas and methane production. In addition, it was concluded that the data obtained should be supported by determining the microorganism counts, feed consumption amounts, and feed efficiency coefficients with *in vivo* studies.

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## Introduction

At the United Nations climate change conference, it was reported that greenhouse gases such as methane, carbon dioxide, nitrous oxide, and ozone, which have heat retaining properties, cause climate change and global warming by absorbing short and long-wavelength solar rays in the atmosphere (Pachauri and Reisinger, 2007; Hook et al., 2010; Kara et al., 2015). Methane, which is one of the greenhouse gases, is defined as a by-product released as a result of the exposure of carbohydrates to the fermentation of microorganisms in the rumen (Flachowsky, 2011; Meale et al., 2012). It has been reported that methane gas released from ruminants shows 23 times more activity in the atmosphere than carbon dioxide gas, and between 80 and 115 million tons of methane gas are emitted into the atmosphere annually by ruminants in the world (Kaya et al., 2012; Joos et al., 2001). It has been noticed that methane gas released from ruminant animals not only causes global warming but also

increases between 3 and 14% of the gross energy depending on the ration content and feed consumption, and up to 15-18% in the case of low-quality roughage feeding. In addition, it has been stated that the methane gas released from ruminant animals not only causes global warming but also causes a loss of 15-18% of the gross energy depending on the ration content and feed consumption (Jouany, 2008; Steinfield et al., 2006). For this reason, in recent years, researchers have focused on reducing the harm caused by methane gas to the environment and increasing the energy use efficiency of ruminant animals. The secondary metabolites they contain in the structure of trees and shrubs are considered toxic to ruminants because they protect the plant against environmental, seasonal and external stimuli. However, in recent years, it has been confirmed that these plant secondary metabolites, which they contain in the leaves, fruits, and stems of trees and shrubs, reduce methane production and show anti-methanogenic properties due to

their effects on the growth rate and fermentation of the rumen microbial population when used in the rations of ruminant animals (Cengiz and Kamalak, 2020; Ku-Vera et al., 2020; Denek et al., 2017; Başer and Kamalak, 2020). Montoya-Flores et al., (2020) reported that TMR containing gladia leaves reduced the methane gas by 20% compared to the control ration in their heifer TMRs, in which they substituted gladia leaves with different tannin content instead of wheat bran. Kamalak and Özkan (2021) reported that sumac leaves with high tannin content can be added into rations to reduce methane gas released by ruminants. Detection of the antimethanogenic effects of tree and shrub leaves on ruminants *in vivo* is a costly and time-consuming process. For this reason, Menke et al. (1979) reported that the anti-methanogenic effects of these feeds could be determined by using a small amount of feed in a short time with the *in vitro* gas production method they developed.

In this study, it was aimed to determine *in vitro* gas and methane production, metabolic energy, net energy lactation and organic matter digestion degrees of ration groups prepared by substituting 10, 20, and 30% sumac leaves (*Rhus coriaria* L.) instead of corn silage in sheep rations.

## Material and Method

The sheep ration, which constitutes the feed material for the study, was obtained from a private farm. The sumac leaves, which were substituted for the corn silage in the sheep ration, were collected from Kahramanmaraş province and from at least five different trees in August 2021. Sheep ration consisting of corn silage (20%), dry meadow grass

(20%), alfalfa straw (22.5%) and commercial feed (37.5%) consisted of control group (K). The rations prepared as iso-nitrogenous and iso-caloric by substituting sumac leaves at 10 (S1), 20 (S2), and 30% (S3) levels instead of corn silage in sheep rations also formed the experimental groups (Table 1). The feeds in the control and experimental groups were dried in an oven at 105°C for 24 hours and ground in a mill with a sieve diameter of 1 mm to be used in chemical and *in vitro* analyses in the laboratory. Dry matter (DM), crude ash (CA), crude protein (CP), and ether extract (EE) contents of feed groups were analysed according to the method reported by AOAC (1990), condensed tannin (CT) contents were analysed according to the method reported by Makkar et al., (1995), and acid detergent fiber (ADF) and neutral detergent fiber (NDF) content were made according to the method reported by Van Soest (1991). Control and experimental group rations were prepared by considering the mixtures of feed raw materials analyzed and the amounts given to the sheep on the farm. DM, CA, CP, EE, NDF, ADF, and CT contents of sumac leaf and corn silage were 93.82 and 94.02%, 7.04 and 8.48%, 8.86 and 10.08%, 5.71 and 3.99%, 28.33 and 66.59%, 11.97 and 33.27%, 0 and 1.33 g/kg, respectively. The ratios of feed raw materials and nutrient compositions in the prepared rations were given in Table 1.

*In vitro* gas and methane production in the control and experimental groups were made according to the reported technique by Menke et al. (1979). The rumen fluid used in the study was obtained from the rumen of 3 rams of the Awassi breed, 2 years old and between 60-70 kg body weight, as reported by Kılıç and Abdiwali (2016).

Table 1. The amount and chemical composition of the prepared ration ingredients

Ingredients (g/kg)	C	S1	S2	S3
Corn silage	200	180	160	140
Alfa alfa straw	225	225	225	225
Dry meadow grass	200	200	200	200
Commercial feed (21% CP)	375	375	375	375
Sumac leaves	-	20	40	60
Chemical composition (%)	C	S1	S2	S3
DM	91.96	92.12	92.16	92.21
CA	7.38	7.35	7.31	7.27
CP	14.14	14.12	14.09	14.07
EE	4.55	4.59	4.62	4.65
NDF	56.43	55.66	54.89	54.11
ADF	28.85	28.42	27.99	27.56
CT(g/kg)	0	0.025	0.053	0.081

DM: Dry matter, CA: crude ash, CP: crude protein, EE: ether extract, NDF: neutral detegent fiber, ADF: acid dergent fiber, CT: condensed tannin

Table 2. In vitro gas, methane, and gas production estimated parameters of Sumac leaf supplemented rations

Groups	GP (ml)	Methane (ml)	Mehtane (%)	ME (Mj/kg DM)	NE <sub>L</sub> (Mj/kg DM)	OMD (%)
C	46.77 <sup>a</sup>	7.48 <sup>a</sup>	15.99	9.41 <sup>a</sup>	5.95 <sup>a</sup>	67.61 <sup>a</sup>
S1	45.26 <sup>a</sup>	7.06 <sup>b</sup>	15.59	9.21 <sup>b</sup>	5.81 <sup>b</sup>	66.24 <sup>b</sup>
S2	43.33 <sup>b</sup>	6.85 <sup>b</sup>	15.69	8.94 <sup>c</sup>	5.61 <sup>c</sup>	64.48 <sup>c</sup>
S3	43.11 <sup>b</sup>	6.80 <sup>b</sup>	15.88	8.91 <sup>c</sup>	5.59 <sup>c</sup>	64.25 <sup>c</sup>
SEM	0.59	0.02	0.06	0.01	0.46	0.01
P	0.000	0.000	0.128	0.000	0.000	0.000

a,b,c: Means within columns with different superscripts differ at P<0.05; C: Control ration; S1: substituted 10% sumac leaves S2: substituted 20% sumac leaves; S3: substituted 30% sumac leaves; SEM: Standard error mean, GP: *in vitro* gas production at 24 h, ME: metabolizable energy, NE<sub>L</sub>= net energy lactation OMD: Digestible organic matter,

The rumen fluid, taken into a screw-capped bottle, was brought to Atatürk University, Agricultural Faculty, Department of Animal Science, Feed Analysis Laboratory with a lidded thermos containing water at approximately 39°C. Rumen liquid was used in the *in vitro* gas production technique after filtering through four layers of cheesecloth by providing an anaerobic environment under CO<sub>2</sub> gas. At the end of the 24th hour of incubation, the produced gas values (GP) were recorded and the methane levels of the released gas were determined using an infrared methane analyzer (Goel et al., 2008). The gas production values (ml) at the end of the 24-hour incubation were corrected according to the Hohenheim weed standard. The ME and OMD values were calculated with the following equations suggested by Menke and Steingass (1988). CP, EE, and CA values in the equations are used as %.

$$\begin{aligned} \text{ME(Mj/kgDM)} &= 2.2 + 0.1357 \times \text{GP} + 0.057 \times \text{CP} + 0.002859 \times \text{EE}^2 \\ \text{NE}_L \text{ (Mj/kg DM)} &= 0.101 \times \text{GP} + 0.051 \times \text{CP} + 0.112 \times \text{EE} \\ \text{OMD} &= 14.88 + 0.8893 \times \text{GP} + 0.448 \times \text{CP} + 0.651 \times \text{CA} \end{aligned}$$

The data were analysed using the SPSS 13.0 (2004) package program and the differences among the group averages were determined by Duncan Multiple Comparison Test (Duncan, 1955).

## Results and Discussion

*In vitro* gas, methane, ME, NE<sub>L</sub>, and OMD values of sheep rations prepared by supplementing sumac leaves in different proportions instead of corn silage are given in Table 2. Sumac leaf supplementation in sheep rations affected all parameters examined significantly (P<0.001).

The *in vitro* gas production values of the rations (ml/200 mg/kg DM) at 24-hour ranged between 43.11 and 46.77, and the highest gas production was found in the C group and the lowest in the S3 group. Gas formed during fermentation occurs in two ways: direct and indirect, and it is known that the amount of gas formed depends on the amount of fermented nutrients. The more fermentable substances are in the feed, the more gas is produced. (Cengiz and Kamalak, 2020). However, it has been reported that the secondary metabolites contained in the leaves of trees and shrubs affect the rate of decomposition of the feed by microorganisms in the rumen and decrease the gas production released as a result of fermentation (Nijjda and Nasiru 2010; Salem et al., 2011; Kılıç and Sarıççek, 2006; Ku- Vera et al., 2020; Ünver et al., 2014). Bhatta et al (2015) found that tannin-containing jack (*Autocarpus integrifolius*), neem (*Azadirachta indica*) and weeping fig (*Ficus bengalensis*) tropical tree leaves reduced the gas production released as a result of fermentation by 42% compared to the control group. It was determined that the 24-hour methane production amounts of the ration groups varied between 6.80 and 7.48 ml. The highest *in vitro* methane production value was found in the control ration, while the lowest value was found in the S3 group. *In vitro* methane gas production values decreased due to increasing doses of sumac bush leaves substituted for corn silage in sheep rations. On the other hand, there was no significant difference between the groups in terms of methane ratios (%) in *in vitro* gas (P>0.05). According to the method determined by Lopez et al., (2010) methane gas values formed in feeds could be classified as low antimethanogenic (>11% and ≤14%), medium antimethanogenic (>6% and <11%) and high

antimethanogenic (>0% and <6%), and by taking these classifications into consideration, the energy use efficiency of ruminants could be increased and the methane gas that causes global warming could be reduced. No antimethanogenic effect was observed in any of the ration groups containing sumac leaves in this study, as reported by Lopez et al., (2010). The metabolic energy contents estimated using the *in vitro* gas values produced for 24 hours in the control and experimental group rations were between 8.91 and 9.41 MJ/kg DM, the net energy lactation contents were between 5.59 and 5.95 MJ/kg DM and the OMD values were between 64.25 and 67.61% determined to have changed. It was determined that the substitution of sumac leaves in sheep rations decreased ME, NE<sub>L</sub>, and OMD values. The highest ME, NE<sub>L</sub>, and OMD values were detected in the control ration, while the lowest was determined in the TMR ration prepared with 30% sumac leave substitute. The values obtained for the metabolic energy, net energy lactation, and organic matter digestion degrees of the rations in the study were found to be higher than the values reported by Boğa et al. (2020) and Kaya and Kaya (2021).

## Conclusion

It has been observed that the substitution of sumac leaves at different rates instead of corn silage in sheep rations has a significant effect on *in vitro* gas and methane production, metabolic energy, net energy lactation, and OMD values (P<0.001). It was determined that there was a decrease in gas production, especially in the groups that substituted 20 and 30% sumac leaves, compared to the control, and metabolic energy, net energy lactation, and OMD values decreased due to the decrease in gas production. On the other hand, the reduction in *in vitro* methane production caused by sumac leaf substitution was not found to be acceptable as an antimethanogenic property. As a result, it was concluded that the secondary compounds in the structure of tree and bush leaves added to the sheep rations should be determined, and the substances that negatively affect the rumen metabolism should be added to the rations by paying attention to the amount of these substances in the ration. In addition, the data obtained from *in vitro* studies should be supported by determining the microorganism counts, feed consumption amounts, and feed efficiency coefficients from *in vivo* studies.

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