

Rumen degradation of dry matter and organic matter digestibility of Cherry tree leaves in ruminant nutrition using *in vitro* gas production and *in situ* techniques

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Abstract: This study was carried out to determine the chemical composition and estimation of nutritional value of cherry tree leaves in the ruminant nutrition. In this study *in vitro* gas production and *in situ* techniques were used to evaluate nutritional value of cherry tree leaves. Cumulative gas production was recorded at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h of incubation also, *in situ* disappear of dry matter for experimental samples was measured at 0, 4, 8, 16, 24, 36, 48, 72 and 96 h of incubation. Chemical composition including dry matter (DM), crude protein (CP), crude Ash (Ash), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF) and tannin compounds, 91.11, 2.76, 9.3, 8, 27.6, 20 and 2.185 percent, respectively measured. Gas production test and *in situ* rumen degradability with of three Taleshi native male cattle rumen fistulae were performed. Digestibility of organic matter (OMD) 65.74 percent and metabolizable energy (ME) 10.27 (MjKg⁻¹) were estimated. The Polyethylene Glycol (PEG) supplementation had also a significant ($p < 0.05$) increase in the estimated parameters of gas production, OMD and ME of samples. Potential degradation (a+b) for dry matter and Effective rumen degradable (ED) at a rate of 0.05/h were estimated, 84.12 % and 52.20% respectively.

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1. Introduction

Tree leaves and shrubs have always played a role in feeding livestock. Trees and shrubs are increasingly recognized as important components of animal feeding, especially as suppliers of protein and energy (FAO, 1997. Gutteridge, 1990. Ikhimioya, 2005).

In difficult environmental conditions, where the available grazing is not sufficient to meet the maintenance requirements of animals for part of the year, the contribution from trees and shrubs is significant. Trees and shrubs are valuable sources of fuel wood, shelter, timber, herbal medicines and food for people, and also help to maintain soil fertility. Tree fodders contain high levels of crude protein and minerals and many show high levels of digestibility. They are readily accepted by livestock and presumably because of their deep-root systems, they continue to produce well into the dry season. However, Anti-nutritive Factors such as polyphenol and tannin compounds can be a problem in some species (Paterson, 1998).

The presence of tannins and other phenolic compounds in a large number of nutritionally important shrubs and tree leaves hampers their utilization as animal feed. High levels of tannins in leaves due to decrease voluntary food intake, nutrient digestibility and N retention (Silanikove, 1996).

There are many method for reduce negative effects of tannins, such as polyethylene glycol (PEG) supplementation. The PEG a non-nutritive synthetic polymer having high binding capacity with tannin compounds (Makkar, 1995), therefore PEG has been widely used to reduce the detrimental effect of tannin compounds in ruminant diets (Makkar, 1995 Pritchard, 1998). There is little information available on the nutritive value of tree leaves in Iran, Therefore the present study was, carried out to determine the chemical composition, phenolic composition and degradation of cherry tree leaves.

2. Material and Methods

Forage Samples During fall season from different parts of East Azerbaijan province were collected. Next, there were drying for one week, and uniform mixture were papered for nutritive chemical. The species of Forage Sample was (*Prunus avium*). For determination of PEG effects, we added PEG with 2:1 ratio (400 mg PEG: 200 mg sample) into gas test syringes.

After drying samples were milled through a 1-mm sieve for chemical analysis. DM was determined by drying the samples at 105°C overnight and ashing the samples in a muffle furnace at 550°C for 6 h. Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude protein was calculated as $N \times 6.25$. Neutral detergent fibre (NDF) and acid

detergent fibre (ADF) contents were determined by the method of AOAC (1990).

Animals: Tree fistulated Taleshi steers (5 years old, about 550 kg weights) were used for rumen application in nylon bag and gas production techniques.

In vitro gas production

Forage samples milled through a 1-mm sieve were incubated in vitro in rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass (1988). Rumen fluid was obtained from three fistulated cattle fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). The samples were incubated in the rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass (Menke, and Steingass, 1988) as follows. 0.200 g dry weight of the sample was weighed in triplicate into calibrated glass syringes of 100 ml in the absence and presence of 400 mg PEG. Syringes were pre-warmed at 39°C before injecting 30 ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39°C. Syringes were gently shaken 30 min after the start of incubation and every hour for the first 10 h of incubation. Gas production was measured as the volume of gas in the calibrated syringes and recorded after incubation of 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours. Total gas values were corrected for blank incubation which contained only rumen fluid. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979).

$$y = a + b(1 - e^{-ct})$$

Where:

a = the gas production from the immediately soluble fraction (ml)

b = the gas production from the insoluble fraction (ml)

c = the gas production rate constant

a + b = the potential gas production (ml)

t = the incubation time (h)

y = the gas produced at the time t

The OMD of forages was calculated using equations of Menke et al.(1979) as follows:

$$\text{OMD (\%)} = 14.88 + 0.889 \text{ GP} + 0.45 \text{ CP} + \text{XA}$$

Where:

GP= is 24 h net gas production (ml / 200 mg),

CP = Crude protein (%)

XA = Ash content (%)

ME (MJ/kg DM) = content of forages was calculated using equations of Menke et al.(1979) as follows:

$$\text{ME (MJ/kg DM)} = 2.20 + 0.136 \text{ GP} + 0.057 \text{ CP} + 0.0029\text{CP}^2$$

Where:

GP = is 24 h net gas production (ml/200 mg)

CP = Crude protein

In situ DM disappearance

In situ nylon bag technique: For determine the *in situ* degradation characteristics of the samples, 4 g of dry sample milled through a 3 mm screen was weighed in nylon bags (16*8 cm, pore size 45 to 60 um). The bags were incubated in the rumen of three fistulated cattle. Nylon bags were withdrawn at 0, 4, 8, 16, 24, 36, 48, 72 and 96 h after insertion. For soluble fraction (0h) measurement was obtained by soaking the two bags of sample in warm water (370°C) for 1 h. The 0 h and incubated bags were then washed with cold water for 15 min in a washing machine and dried for 48 h at 60°C. The DM degradation data were fitted to the exponential equation $p = a+b(1-e^{-ct})$ (Ørskov and McDonald, 1979).

To determine the degradation characteristics (a, b, a+b, c, ED); where p is the DM degradation at time t, a denotes washing loss (representing the soluble fraction of the feed); b insoluble fraction; c is the rate of degradation of fraction b; ED denotes effective degradability, calculated at an outflow rate of (0.02, 0.05 and 0.08 per h). After incubation, DM degradability (DMD) for each bag, for each incubation period and for each cattle were calculated with formulas suggested by Susmel et al. (1990)

Where:

y = DM disappearance in rumen at time t

a = the rapidly soluble fraction

b = the potentially degradable fraction

c = the constant rate of degradation of b (%/h)

Effective DM Degradability (EDMD) was calculated applying the equation of Orskov and McDonald (1979).

Statistical Analysis

All of data were analysis by using SAS software (1999) and means of two sample groups were separated by independent samples t-test (Torrie JH, 1980). All data obtained from three replicates (n= 3).

3. Results and discussion

The chemical composition of Cherry leaves has shown in table1

Chemical composition including dry matter (DM), crude protein (CP), crude Ash (Ash), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF) and tannin compounds, 91.11, 2.76, 9.3, 8, 27.6, 20 and 2.185 percent, respectively measured.

Table 1: The chemical composition of cherry tree leaves (%)

Dry matter	91.11
ether extract	8
Crude protein	2.76
Neutral detergent fiber	27.6
Acid detergent fiber	20
Ash	9.30
tannin compounds	2.185

***In vitro* gas production**

Gas production volumes (ml/200mg DM) at different incubation times has shown in figure 1.

There are a steadily increase in the gas production for over a period of 24h.

The gas production parameters, are given in Table 2. The soluble fraction (a) and insoluble but fermentable fraction (b), for with PEG and without PEG treatments were -1.44, 54.38 and -4.25, 52.90 ml, respectively. The negative (a) value for both treatments due to delay in onset of fermentation and microbial attachment were in agreement with Chumpawadee et al (2005) and Maheri-sis et al (2008). The PEG supplementation increased the gas production from the gas production of immediately soluble fraction (a) and gas production from the gas production rate (c), Whereas PEG supplementation had no significant effect on the gas production from insoluble fraction (b) and the potential gas production (a+b), also there were significant increases ($P < 0.05$) in the OMD and ME content of the Cherry leaves in the addition of PEG.

***In situ* degradability characteristic of apple leaves shown in table 3.**

The DM degradability from the soluble fraction (a) and Potential degradability (a+b) were, 14.32 and 84.12 percent respectively and the rate degradability(c) was 0.060/h estimated. The total degradability of the sample is given by a + b which obviously cannot exceed 100. It follows that 100 - (a+b) represents the fraction which will appear to be undegradable in the rumen. If 'a' is positive, then there is a component which is degraded rapidly and/or a component which is soluble, or fine enough to escape from the bags simply by soaking and washing. Whether 'a' represents rapid degradation, or simply washing losses, can be determined with control bags which are simply soaked in water and then washed and dried in the normal way. When a negative value for 'a' is obtained this means that there has to be an initiation period for degradation to start (termed the lag phase).

Effective degradability (ED) of the examined nutrient components were calculated using the outflow rates of 0.02, 0.05 and 0.08/hr, according to Ørskov et al. (1980), model: $ED = a + [bc/(c+k)]$ where ED is effective degradability and 'a', 'b' and 'c' are the constants as described earlier in the non-linear equation above and 'k' the rumen fractional outflow rates.

Effective rumen degradable dry matter at a different rate were determine, Effective degradability (ED) of DM decreased with increase in outflow rates DMD decreased of 66.46% ($k=0.02$) to 44.13% ($k=0.08$) in the leaves.

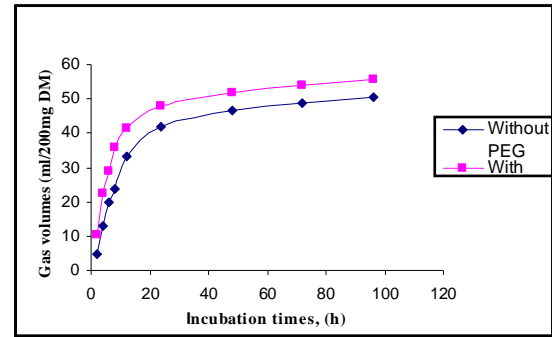


Fig1. *In vitro* gas production volume of cherry tree leaves at different incubation time in the presence Of (PEG)

Table 2. The estimated parameters from the gas production of cherry tree leaves.

Estimated Parameters	Without PEG	With PEG	P value	SEM
a	-4.25 ^b	-1.44 ^a	$P < 0.0001$	0.201
b	52.90	54.38	$P < 0.022$	0.530
a +b	57.15	55.82	$P < 0.026$	0.680
c	0.097 ^b	0.135 ^a	$P < 0.0001$	0.005
OMD	62.74 ^b	68.14 ^a	$P < 0.003$	0.402
ME	10.27 ^b	11.10 ^a	$P < 0.003$	0.02

a: the gas production from soluble fraction (ml/200mg DM)

b: the gas production from insoluble fraction (ml/200mg DM)

c: rate constant of gas production during incubation (ml/h)

(a + b): the potential gas production (ml/200mg DM),

OMD: Organic matter digestibility (%),

ME: Metabolisable energy (MJ/kg DM), and

S.E.M: standard error of the mean.

Table 3. The parameters of estimated from the *In situ* degradability of cherry leaves (%)

Cherry tree leaves DM	Estimated Parameters			
	a	b	a+b	c
	14.32	69.79	84.12	0.060
effective degradability				
	Lag time	0.02	0.05	0.08
DM	0.782	66.46	52.20	44.13

a = washing losses, soluble or rapidly degradable:

This value is the intercept of the degradation curve at time 0 h (%)

b = the insoluble but potentially fermentable (%)

c = degradability rate (h)

a+b = Potential degradability (%)

Conclusion

In conclusion, this study has demonstrated that the use of cherry leaves, which have low crude protein (about 2.76% of DM) therefore, recommended the use of these resources be used to supplement protein

in ruminant diets also, the high percentage of gas production, dry matter degradation and low percentage of tannin compounds having tested samples, we can say that the cherry tree leaves have relatively good nutritional value in ruminant nutrition. However, further study is needed to investigate of use of tree leaves in ruminant diets.

PEG supplementation had a significant increased ($P<0.05$) on the gas production, OMD and ME content of cherry tree leaves.

PEG addition, significant increased ($P<0.05$) the gas volumes in all different incubation times, gas production from soluble fraction (a), and gas production rate (c), but had no effect on the gas production from the insoluble fraction (b) and the potential gas production (a+b).

PEG supplementation to improve the nutritive value of tannin-containing tree leaves.

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