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Investigation of nutritional value of Aprical Tree Leaves in the presence of Polyethylene Glycol (PEG)

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ABSTRACT

The aim of present study was to determine the chemical composition and to estimate the nutritive value of Aprical tree leaves as feedstuffs for ruminants, after addition polyethylene glycol (PEG). Experimental materials were collected from different parts of Eastern Azerbaijan province (northwestern Iran). After drying the samples and provide uniform mix, chemical composition including dry matter (DM), crude protein (CP), ether extract (EE), crude ash (CA), neutral detergent fiber (NDF), acid detergent fiber (ADF), polyphenol and tannin compounds were estimated; 93.11, 3.54, 6, 15, 29.2, 20.8, 1.55 and 0.617 percent, respectively. Gas production test with mixtures of filtered rumen liquid of two Taleshi native male cattle rumen in time periods of 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours were performed. Because of tannins content of experimental samples, we added PEG with 2:1 ratio (400 mg PEG: 200 mg sample) into gas test syringes, for evaluation of PEG effects. The PEG supplementation had also a significant ($p<0.05$) increase in the estimated parameters of gas production, Organic matter digestibility (OMD) and Metabolizable energy (ME) of Aprical tree leaves. Based on the obtained results it is concluded that the PEG supplementation reduce of tannins negative effects of Aprical tree leaves and suggested that the Aprical tree leaves has relatively good nutritional value for ruminant.

Key words: Chemical composition, Aprical leaf, Polyphenol compounds, Gas production, Metabolizable energy, Organic matter digestibility.

INTRODUCTION

Shrub and tree leaves are an important component of diets for goats, cattle and sheep [1], and play an important role in the nutrition of grazing animals [2]. The utilization of this resource

is limited by the high lignin content and the presence of anti-nutritional factors such as polyphenol and tannin compounds. 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 [3]. High levels of tannins in leaves decrease voluntary food intake, nutrient digestibility and N retention [4,5]. There are many method for reduce of tannins negative effects, such as polyethylene glycol (PEG) supplementation. The PEG a non-nutritive synthetic polymer having high binding capacity with tannin compounds [6], therefore PEG has been widely used to reduce the detrimental effect of tannin compounds in ruminant diets [7]. Tannins have beneficial effects in Rumen Environment, suppression of bloat [8], and increase rumen undegrable protein (RUP) via increase feed proteins resistanting [9]. Overall according to many research about tannins, It seem a level of this resource below 5% to be tolerable for ruminants. In vitro gas production [10] has been used to assess the nutritive value of feedstuffs; these rapid and less expensive methods have been used to screen feed resources before making them available to livestock. The objective of this study was to determine the chemical composition and assess the effect of PEG addition on in vitro gas production kinetics, Organic matter digestibility (OMD) and Metabolizable energy (ME) of Aprical Tree Leaves.

MATERIALS AND METHODS

2.1. Forage Samples: During fall season forage samples were collected from different parts of Eastern Azerbaijan province. Next, there were drying for one week, and uniform mixture were papered for nutritive chemical .The species of Forage Sample was *Prunus armanica*. For determination of PEG effects, we added PEG with 2:1 ratio (400 mg PEG: 200 mg sample) [11], into gas test syringes. All samples were then ground in a laboratory mill through a 1 mm screen.

2.2. Chemical Analysis

Dry matter (DM) was determined by drying the samples at 105°C overnight and ash by igniting the samples in a muffle furnace at 550°C for 6 h. Nitrogen (N) content was measured by the Kjeldahl method [12]. Crude protein was calculated as N X 6.25. Acid detergent fiber (ADF) content and neutral detergent fiber (NDF) content of leaves were determined using the method described by Van Soest *et al.* [13]. Non-Fibrous Carbohydrate (NFC) is calculated using the equation of NRC [14], NFC = 100 – (NDF + CP + EE + Ash). Condensed tannin was determined by butanol-HCl method as described by Makkar *et al*. [15]. All chemical analyses were carried out in triplicate.

2.3. In vitro gas production

Rumen fluid was obtained from two 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 [10] 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 [16].

$y= a + b (1-exp^{-ct})$
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 for the insoluble fraction (ml/h)
t = incubation time (h)
y = gas produced at time 't'

The OMD of forages was calculated using equations of Menke *et al.* [17] as follows:

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

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.* [17] as follows:

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

Where:
GP is 24 h net gas production (ml/200 mg),
CP = Crude protein

2.3. Statistical Analysis
All of data were analysis by using software of SAS [18] and means of two sample groups were separated by independent samples t-test [19]. All data obtained from three replicates (n = 3).

RESULTS AND DISCUSSION

3.1. Chemical composition
The chemical composition of Aprical Tree Leaves shown in Table 1. Chemical composition including dry matter (DM), crude protein (CP), ether extract (EE), crude ash (CA), neutral detergent fiber (NDF), acid detergent fiber (ADF), polyphenol and tannin compounds were estimated; 93.11, 3.54, 6, 15, 29.2, 20.8, 1.55 and 0.617 percent, respectively. The polyphenol and tannin compounds concentration in the Aprical tree leaves were lower in this study.

3.2. In vitro gas production
Gas production volumes (ml/200mg DM) at differents incubation times shown in Figure1. There are a steadly increase in the gas production for over a period of 24h.

The gas production kinetics, are given in Table 2. There are considerable increases in gas production when the Aprical leaves were incubated in the addition of PEG.

The gas volumes in addition of PEG in different incubation times were higher than without PEG treatment. The soluble fraction (a) and insoluble but fermentable fraction (b), for with

PEG and without PEG treatments were -6.81, 58.54 and -6.54, 54.92 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 and Maheri-sis et al [20,21]. The PEG supplementation increased the gas production from the gas production of insoluble but fermentable fraction (b), potential gas production (a+b) and gas production from the gas production rate (c), Whereas PEG supplementation had no significant effect on the gas production from the immediately soluble fraction (a), also there were significant increases ($P<0.05$) in the OMD and ME content of the Aprical leaves in the addition of PEG. These results are in agreement with the findings of Getachew et al. [22,23] and, Seresinhe and Iben [24] and findings of kiyani et al [1]. The increase in the gas production in the presence of PEG is possibly due to an increase in the available nutrients to rumen micro-organisms, especially the available nitrogen. [25] Showed that addition of PEG caused a significant and marked increase in the rate and extent of ammonia production.

The mechanism of dietary effects of tannins may be understood by their ability to forming complex with proteins. Tannins may formed a less digestible complex with dietary proteins and may bind and inhibit the endogenous protein, such as digestive enzymes [26]. Tannin can adversely affect the microbial and enzyme activities [27,28]. The improvement in gas production, OMD and ME with PEG emphasizes the negative effect tannins may have on digestibility. The results of this experiment support the fact that PEG can be added to tannin-containing plant material in *in vitro* fermentation systems to demonstrate the nutritional importance of tannins on organic matter digestibility and to measure nutritive value of the forage after neutralization [15,23]. However there is a lack of information about feasibility of using PEG in tannin-rich diets for ruminants. PEG supplementation to improve the nutritive value of Aprical leaves should be further analyzed in detail whether or not it is economical due to high price of PEG, before large scale implementation. However, Makkar [29] reported that some other substances such as wood ash, NaOH and urea can be used instead of PEG.

Table 1 .The chemical composition of aprical tree leaves (%)

Dry matter	ether extract	Crude protein	Neutral detergent fiber	Acid detergent fiber	Ash	Polyphenolic compounds	Condensed tannin	Nonfibrous carbohydrates
93.11	6	3.54	29.2	20.8	15	1.51	0.61	46.26

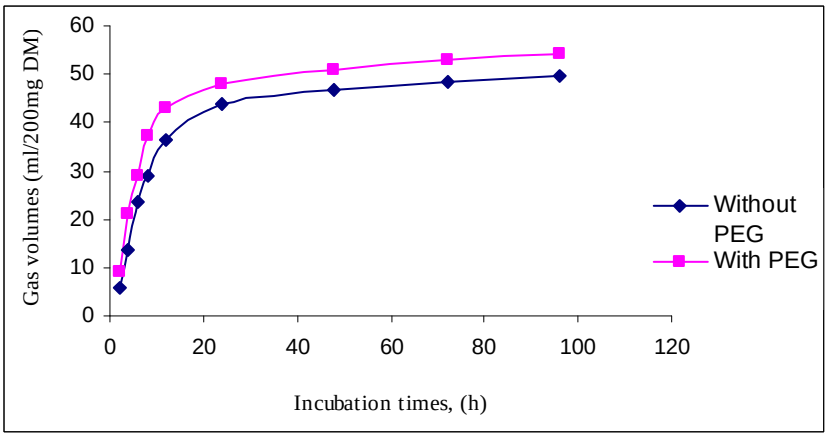

Fig. 1. *In vitro* gas production volume of aprical tree leaves at different incubation time in the presence of (PEG)

Table 2. *In vitro* gas production volumes (ml/200mg DM) of aprical tree leaves at different incubation times

	Incubation times								
Treatment	2	4	6	8	12	24	48	72	96
Without PEG	5.83	13.84	23.73	28.98	36.39	43.96	46.56	48.98	50.41
With PEG	9.29	21.25	28.88	37.04	43.17	47.88	50.95	52.87	54.41
P value	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.003	P<0.001	P<0.009
SEM	0.144	0.148	0.189	0.256	0.349	0.452	0.465	0.392	0.664

Table 3. The estimated parameters from the gas production of aprical tree leaves.

Treatment	Estimated Parameters					
	a	b	| a | +b	c	OMD	ME
Without PEG	-6.54	54.92	61.74	0.128	70.56	10.85
With PEG	-6.81	58.54	65.08	0.160	74.24	11.41
P value	P<0.366	P<0.008	P<0.026	P<0.001	P<0.003	P<0.003
SEM	0.201	0.530	0.680	0.005	0.402	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

CONCLUSION

- PEG supplementation had a significant increased (P<0.05) on the gas production, OMD and ME content of Aprical tree leaves.
- PEG addition, significant increased (P<0.05) the gas volumes in all different incubation times, gas production from insoluble fraction (b), potential gas production (a+b) and gas production rate (c), but had no effect on the gas production from the immediately soluble fraction (a)
- PEG supplementation to improve the nutritive value of tannin-containing tree leaves
- The improvement in gas production, OMD and ME with PEG emphasizes the negative effect of tannins on digestibility.

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