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Effect of Polyethylene Glycol (PEG) on *in vitro* Gas Production, Metobolizable Energy and Organic Matter Digestibility of Apple Tree Leaves as Ruminant Feed[1]

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Abstract: This study was carried out to determine the chemical composition and to estimate the metabolizable energy and digestible organic matter of apple tree leaves as ruminant feed, after addition with polyethylene glycol (PEG). Experimental materials from different parts of Eastern Azerbaijan province (northwestern Iran) were collected. After drying the samples and provide uniform mix, chemical composition including dry matter (DM), crude protein (CP), ether extract (EE), crude ash (CA), cell wall (NDF) and cell wall without hemicellulose (ADF) were estimated as 92.53, 10.80, 9, 8.60, 23 and 15.4 %, respectively. Gas production test with mixtures of filtered liquid of two Taleshi native male cattle rumen after 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours was performed. The amount of gas produced after 24 and 96 hours incubation, was equivalent to 52.44 and 62.60 ml per 200 mg dry matter of sample, respectively. The digestibility of organic matter was 74.96% and the metabolizable energy was 11.48 ($MjKg^{-1}$). 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, OMD and ME of samples. Based on the obtained results it is concluded that the apple tree leaves has relatively good nutritional value for ruminant nutrition.

Key words: Chemical composition · Apple leaf · Gas production · Metabolizable energy · Organic matter digestibility

INTRODUCTION

In developing countries, with increasing of protein requirements of developing population, animal feeding with use of cheap foodstuffs is so important factor.

Interest in use of tree and shrub species as sources of fodder for livestock has been increasing in recent years particularly in tropical and subtropical countries [1]. Tree leaves are an important component of diets for goats, cattle, deer, game and sheep [2, 3] and play an important role in the nutrition of grazing animals in areas whereas few or no alternative feed are available [4]. 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 [5]. High levels of tannins in leaves restrict the nutrient utilization and decrease voluntary food intake, nutrient digestibility and N retention [6-8].

Tannins act within the animal's digestive tract by binding to the substrate to be digested (usually proteins, carbohydrate, lipids) and inhibiting digestive enzymes or exerting anti-microbial effects [9]. However, PEG can form a stable complex with tannins thereby prevent the binding of tannins with proteins (10). Therefore, PEG has been widely used to reduce the detrimental effect of condensed tannin in ruminant diets [11-14].The aim of this study was to determine the effect of PEG on *in vitro* gas production kinetics, OMD and ME of Apple leaves.

MATERIALS AND METHODS

Forage Samples: During fall season, forage samples were collected from different parts of East Azerbaijan province. Next, there were drying for one week and homogeneous mixture were papered for nutritive chemical

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analyzes. For determination of PEG effects, we added PEG with 2:1 ratio (400 mg PEG: 200 mg sample) into gas test syringes.

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 [15]. 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.* [16]. Condensed tannin was determined by butanol-HCl method as described by Makkar *et al.* [17]. All chemical analyses were carried out in triplicate.

***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 [18] 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. The 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. The 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 was recorded before incubation (0) and 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours after incubation. 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 [19].

$$y = a + b\,(1-\exp^{-ct})$$

Whereas:

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 (b)
t = Incubation time (h)
y = Gas produced at time 't'

The OMD of forages was calculated using equations of Menke *et al.* [20] as follows:

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

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.* [20[as follows:

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

Whereas:

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

Statistical Analysis: One-way analysis of variance (ANOVA) was carried out to compare gas production kinetics, OMD and ME values using the General Linear Model (GLM) of Statistics for windows. Significance between individual means was identified using the T-tests. Mean differences were considered significant at ($P<0.05$). Standard errors of means were calculated from the residual mean square in the analysis of variance

RESULTS AND DISCUSSION

The chemical composition of Apple Tree leaves is given in Table 1.

There was a considerable increase in gas production when the apple leaves were incubated with the addition of PEG (Figure 1). This result is in agreement with findings of Seresinhe and Iben [21] and Tedonkeng *et al.* [22].

The gas production kinetics, are given in Table 2. The PEG supplementation had also a significant ($p<0.05$) effect on the estimated parameters of OMD and ME (Table 2). PEG supplementation increased the gas production rate (c) whereas PEG supplementation had no

Table 1: The chemical composition of *apple tree* leaves

Constituents	g/kg
Dry matter	925
As g/kg of DM	
Crude protein	108
Neutral detergent fiber (NDF)	230
Acid detergent fiber (ADF)	158
Ash	86
Polyphenolic compounds	62
Condensed tannin	16

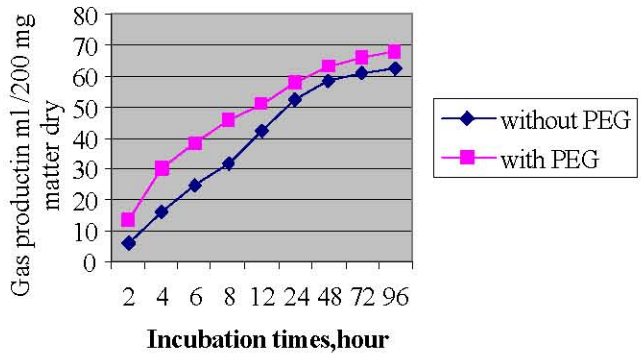

Fig. 1: The effect of Polyethylene glycol on gas production

Table 2: The gas production of *apple* leaves

	Incubation times								
Treatment	2	4	6	8	12	24	48	72	96
Without PEG	6.21[b]	16.12[b]	24.90[b]	31.50[b]	42.41[b]	52.44[b]	58.48[b]	61.20[b]	62.60[b]
With PEG	13.64[a]	30.11[a]	38.03[a]	45.66[a]	51.17[a]	58.08[a]	63.15[a]	66.00[a]	68.00[a]
SEM	0.134	0.145	0.134	0.112	0.148	0.184	0.186	0.188	0.188
P value	P<0.0001								

Table 3: The parameters estimated from the gas production of apple leaves

	Estimated Parameters					
Treatment	a	b	a+b	c	OMD	ME
Without PEG	5.65[a]	66.25[a]	71.90[a]	.101[b]	74.96[b]	11.48[b]
With PEG	1.08[b]	65.48[a]	66.56[b]	.147[a]	79.95[a]	12.24[a]
SEM	0.320	0.455	0.360	0.003	0.607	0.092
P value	P<0.0001					

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 (b),
a+b: Potential gas production,
ME: Metabolizable energy, OMD: Organic matter digestibility

effect on the gas production from the immediately soluble fraction (a) and insoluble fraction (b), On the other hand, there were significant ($p<0.05$) increases in the OMD and ME content of the apple leaves. These results are in agreement with the findings of Getachew *et al.* [23], Getachew *et al.* [24] and, Seresinhe and Iben [21]. PEG also can liberate protein from the preformed tannin-protein complexes [25]. 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. McSweeney *et al.* [26] 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 [27].Also, tannin can adversely affect the microbial and enzyme activities [28 -30]. The improvement in gas production, OMD and ME with PEG emphasizes the negative effect that tannins may have on digestibility. PEG, a non-nutritive synthetic polymer, has a high affinity to tannins and makes tannins inert by forming tannin PEG complexes [17]. PEG can also liberate protein from the preformed tannin-protein complexes [25]. PEG had a significant effect on OMD and ME contents of apple leaves.

The results of this experiment supported 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 [17, 23, 26]. 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 apple 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 [32] reported that some other substances such as wood ash, NaOH and urea can be used instead of PEG.

CONCLUSION

- PEG supplementation had a significant effect on the gas production, OMD and ME content of apple tree leaves.
- PEG supplementation could be used 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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