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Deactivation of tannins in raisin stalk by polyethylene glycol-600: Effect on degradation and gas production *in vitro*

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An experiment was conducted to assess the effects of polyethylene glycol (PEG-6000) and urea on dry and organic matter digestibility (IVDMD and IVOMD, respectively) and gas production *in vitro*. Raisin stalk contained 8.6% crude protein, 85% dry matter, 7% ash, 13.95% total extractable phenol (TEPH) and 2.13% total extractable tannin (TET). The experimental treatments were: raisin with no supplementation as control (C); supplemented with 3% urea (U 3%), 5% urea (U 5%) or 3% urea plus 5% PEG (U-PEG) per DM. The rumen liquor fluid was obtained from two ruminal-cannulated fat-tailed sheep. TEPH and TET were determined and *in vitro* incubation was also conducted. The results indicated that the PEG increased IVOMD and IVDMD. The gas produced from time 0 to 3 and 3 to 6 h of incubation were significantly higher in PEG treatment than that of other groups (P < 0.05). The U-PEG treatment increased the total gas production in 96 h, but it was not statistically significant (P = 0.06). Gas production in 96 h incubation using 300 mg fresh sample was significantly higher than 200 mg sample (P < 0.05). It is concluded that the negative effect of tannin on DM and OM digestibility and also gas production of raisin stalk *in vitro* could be alleviated by PEG treatment.

Key words: Raisin stalk, tannin, polyethylene glycol (PEG), urea, gas production, digestibility, *in vitro*.

INTRODUCTION

Tannins are a heterogeneous group of phenolic polymers of the plant origin that can be detrimental when consumed by herbivores (Villalba and Provenza, 2002). This negative effect is attributed to the defense mechanism of the plant to inhibit the action of predators. Tannins play a role in protecting plants from herbivory and disease. Tannins have been divided into two classes based on their chemical structures: condensed and hydrolysable tannins (Silanikove et al., 2001). Moderate levels of tannins can have beneficial responses in ruminants (Min et al., 2003; Hove et al., 2001), however, the presence of high level of tannins in feed stuffs often limits the digestibility of the diet by their ability to combine with proteins that is stable at rumen pH (Frutos et al., 2004). Tannins can also inhibit enzymatic (Ben et al., 2000) and microbial activity (McSweeney et al., 2001). The anti nutritive effects of tannins are associated with their ability to com-

bine with dietary proteins and polymers such as cellulose, hemicellulose, pectin and minerals, thus retarding their digestion (McSweeney et al., 2001). Raisin stalk is a by-product of the raisin industry. There is paucity of information available on the nutritive value of raisin stalk in animal nutrition. Raisin stalk contained 8.6% crude protein, 85% dry matter, 7% ash, 13.95% total extractable phenol and 2.13% total extractable tannin (Tabatabaei et al., 1994). The nutritive values of raisin stalk are limited by the presence of anti nutritional factors such as tannins. Salam et al. (2005) and Landau et al. (2003) suggested that activated charcoal, urea and polyethylene glycol 400 treatments are efficient in decreasing phenolic com-pounds in acacia leaves. Polyethylene glycol binds to tannins and may thereby increase the availability of certain macronutrients, particularly of proteins (Makkar, 2003). Markantonatos (1992) reported higher rate of gas production using Mediterranean Browses containing a relatively low concentration of total extractable phenols. These findings were consistent with other studies (Makkar, 2003; Baba

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Table 1. Experimental treatment (g/kg DM).

Ingredient	Treatment			
	C	U 3%	U 5%	U-PEG
Raisin stalk	999.3	932.6	885.4	847
Urea	-	27.2	44.2	42.3
Molasses	-	37.7	68.2	65.2
PEG	-	-	-	43.4

RS, Raisin stalk (control); U 3%, raisin stalk treated with 3% urea; U 5%, raisin stalk treated with 5% urea; U-PEG, raisin stalk treated with 3% urea; 5% polyethylene glycol.

Table 2. Chemical composition of experimental treatments (%DM).

Parameter	C	U 3%	U 5%	U-PEG
DM	72.66	73.29	73.68	74.53
OM	93.05	93.15	93.22	89.24
CP	8.6	16.18	20.55	19.65
Ash	6.95	6.85	6.78	10.76
TEPH [1]	13.9			
TET [2]	2.13			

[1]Total extractable phenol-percentage of acid tannic content (DM); [2]total extractable tannin-percentage of acid tannic content (DM)

et al., 2002). Similarly, Frutos et al. (2002) reported that plant with higher than 176 g *quebracho* tannin equivalents/ kg DM were negatively correlated ($P < 0.05$) with OM degradation and cumulative gas production.

More direct methods for deactivation of tannins are biological ways (white-rot fungi) and chemical ways such as precipitants (polyethylene glycol and polyvinyl pyrolidone), alkali (example, sodium or potassium hydroxide) (Makkar, 2003; Baba et al., 2002; Ben et al., 2005) and physical drying (Ben et al., 1999*)*. Polyethylene glycol, as a tannin-complex agent can binds to tannins and thus neutralizes their negative effects (Palmer and Jone, 2000). The objective of this study was to evaluate the effect of PEG 6000 and urea *in vitro* in dry matter and organic matter digestibility, and gas production of raisin stalk.

MATERIALS AND METHODS

This study was performed at Razi University and Research center of Tabriz University, during July and August, 2007. Four experimental treatments: raisin stalk with no supplementation (C), 3% urea (U3%), 5% urea (U5%) or 3% urea plus 5% PEG (U-PEG) were used (Table 1). The crude ash, dry matter, crude protein and organic matter of raisin stalk were analyzed as described by AOAC (1990). Total extractable phenol (TEPH) were determined according to the method described by Julkumen-Tiitto (1985) using the phenol ciocalteau (Folin reagent). Total extractable tannins (TET) were estimated indirectly after being absorbed to insoluble polyvinyl pyrrolidone (PVP) according to Makkar et al. (1992).

In vitro incubation was conducted according to the procedures of Menke and Steingass (1988) using 30 ml buffered rumen inocu-

lums. The rumen liquor was obtained from two ruminal-cannulated sheep receiving diet of alfalfa hay. Dietary samples were milled in 1 and 3 mm screen. The samples were then weighed in two levels of 200 ± 5 and 300 ± 5 mg, into calibrated glass syringes. Three syringes containing 30 ml inoculums (without sample) served as the blanks. The syringes were incubated in a water bath maintained at $39 \pm 0.1°C$, and were gently shaken every one hour during the first 8 h of incubation. Readings were recorded at 3, 6, 12, 24, 48, 72 and 96 h incubation. After incubation for 96 h, the residues were washed by 20 ml buffer liquor and 20 ml distilled water. The residues was then weighed and analyzed for DM and ash by AOAC (1990). Dry matter was determined by drying the sample at 80°C for 24 h. The OM was determined after burning at 550°C for 7 h. Dry matter and organic matter digestibility were calculated from the initial and final weights of DM and OM, after corrections using data from the blank tubes. After incubation, T/2 (time to half-asymptote) was determined. An estimate of the efficiency of fermentation partitioning factor (PF) was calculated. Partitioning factor of substrate to gas, which was expressed as mg DM degraded/ml gas and reflect the variation in microbial biomass yield. PF = truly DM degraded/ total gas production.

The data was analyzed using the GLM procedure and the Minitab according to completely randomized design. The Tukey test was applied to determine the significance between treatment means.

RESULTS AND DISCUSSION

The raisin stalk had a relative low concentration of CP, while the CP in 3 and 5% urea treatments averaged 16.18 and 20.55% (DM), respectively (Table 2). Urea treated raisin stalk, increased CP from 8 to 20.55 in treatment (U 5%). The TEPH and TET (acid tannic/DM raisin stalk) were 139 and 21.3 g/kg, respectively (Table 2). The PEG treatment increased IVDMD and IVOMD from 62.15

Table 3. The effect of urea and PEG on *in vitro* digestibility (T/2 and PF).

Parameter	Treatment				SEM
	C	U 3%	U 5%	U-PEG	
IVDMD (%)	62.15	70.56	80.28	81.25	3.05
IVOMD (%)	68.4	74.35	78.84	84.59	3.64
T/2 (h)	8.59	8.95	9.18	7.13	
PF (mg/ml)	2.28	2.53	2.96	2.54	
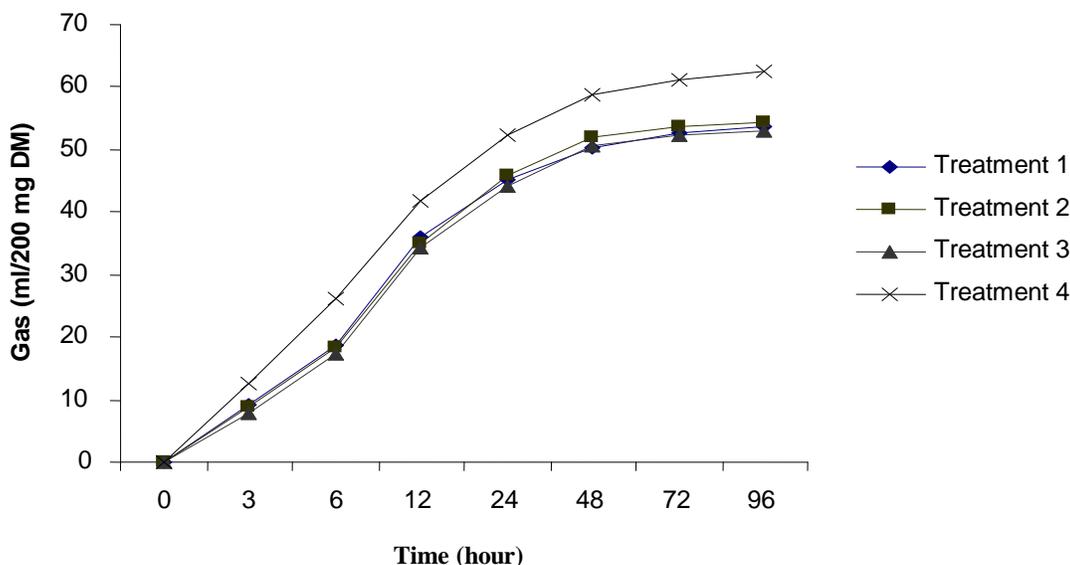
Figure 1. Cumulative gas production profiles from the fermentation of raisin stalk. (♦): C, (■): U 3%, (▲): U5%, (×): U-PEG.

to 81.25%. Similarly, the addition of urea increased the amounts of IVDMD and IVOMD in comparison with control group, although these differences were not significant (P > 0.05). The amount of T/2 in UPEG diet was shorter than those of the other groups (Table 3). The differences in IVDMD and IVOMD measured in this experiment are similar with other *in vitro* results (Palmer and Jones, 2000a, b). In Palmer and Jones (2000) study, the ground samples had the highest PEG binding and so, had the greatest opportunity to overcome negative effects of tannins in IVDMD.

Cumulative gas production profiles from the *in vitro* fermentation of raisin stalk ranged from a minimum value (53.14 ml) in the third group (U 5%) to maximum value (62.47 ml) in UPEG treatment (Figure 1). PEG increased total gas production in 96 h., but it was not statistically significant (P = 0.06). In addition, TEPH and TET were negatively correlated with gas production but there was no significant correlation between all treatments. Urea treatments of raisin stalk did not positively affect the gas production.

The amount of gas produced in different times of incubation was significant for gas production during 6 h

(Figure 2). The highest rate of gas production was recorded in UPEG group (0 to 3 and 3 to 6 h). There were no significant differences between treatments in 24 to 96 h incubation. The PEG treatment in 0 to 6 h incubation gave markedly higher value than the other treatments (P < 0.05). The addition of PEG-6000 to the incubation medium increased the gas production (Figure 1). The gas production parameters were comparable with those reported for other tanniniferous plants (Ben et al., 2005).

The amount of gas production changed using different size of samples after 96 h incubation. Small size of the samples (1 mm) had more gas production from diet ground to pass a 3 mm sieve (Figure 3). Increasing the amount of sample from 200 to 300 mg significantly increased the amount of gas production in all treatments (P < 0.05).

Studies of *in vitro* and *in situ* degradation have been shown to have a positive response to incubation of the tannin-containing plants samples with tannin-binding agents such as PEG in comparison to non-treated samples (Gatechew et al., 2001). Abdulrazak et al. (2000) reported that the rate of gas production of *Acacia nubica* was 42.6 ml/200 mg DM/ with lowest TEPH and
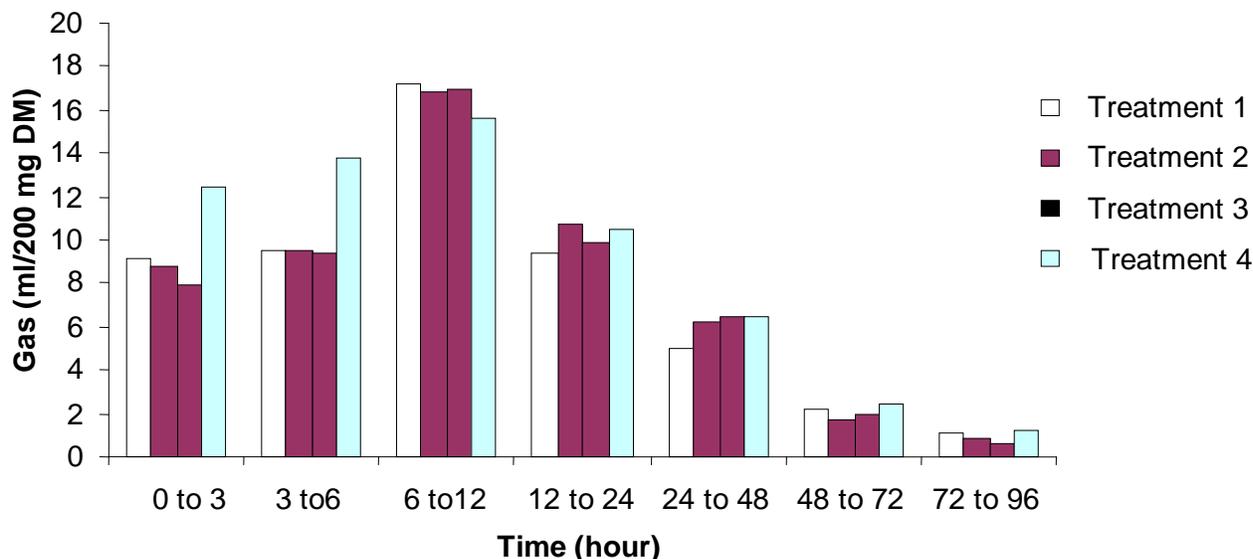
Figure 2. The effect of urea and PEG on cumulative gas production profiles from the fermentation of raisin stalk. T1: C; T2: U 3%; T3: U5%; T4: U-PEG.
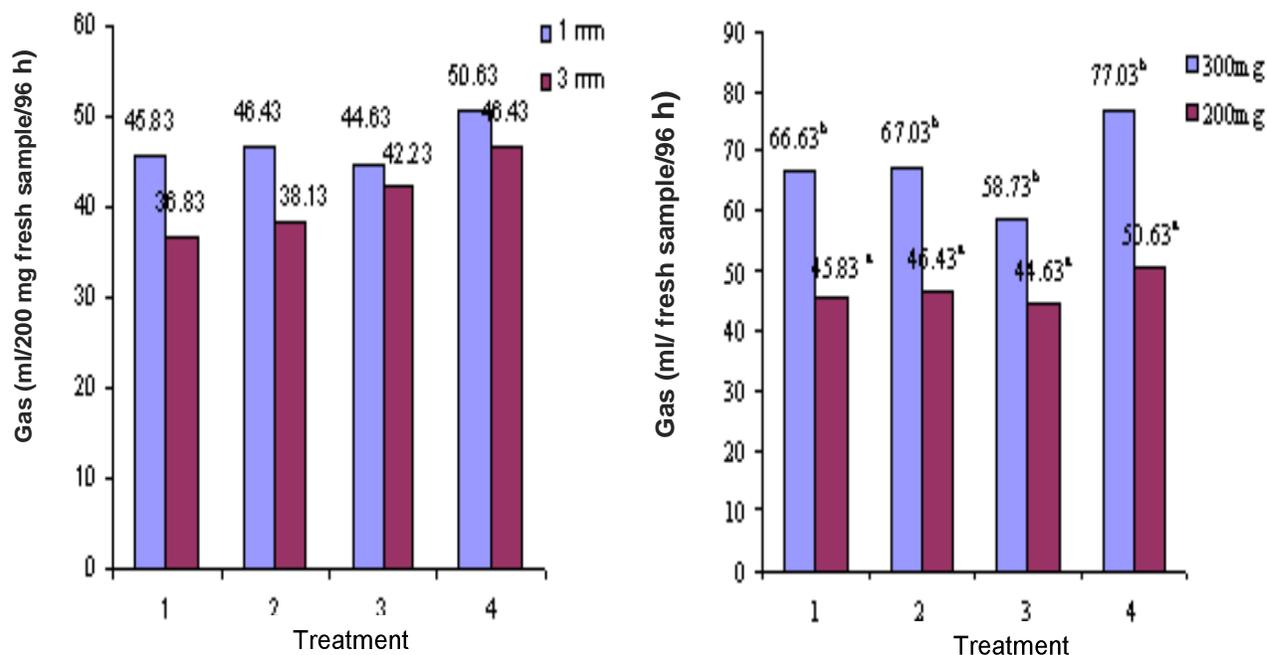

Figure 3. The effect of amount and size of sample on cumulative gas production profiles from the fermentation of raisin stalk. 1: C; 2: U 3%; 3: U 5%; 4: U-PEG.

TET (56 and 39 mg/g DM, respectively), which significantly ($P < 0.05$), was higher than other groups in 96 h incubation. Markantonatos (1992) reported higher rate of gas production using Mediterranean Browses containing a relatively low concentration of total extractable phenols. These findings were consistent with other studies (Makkar, 2003; Baba et al., 2002). Similarly, Frutos et al.

(2002), reported that plant with higher than 176 g *quebracho* tannin equivalents/ kg DM were negatively correlated ($P < 0.05$) with OM degradation and cumulative gas production.

In this study, PEG treatment increased the gas volume when compared to other treatments. Charcoal, urea and polyethylene glycol 400 treatments are efficient in

decreasing phenolic compounds of Acacia leaves (Salam et al., 2005). Supplementing tannin-containing sample with PEG-6000 increased gas produced from 45 ± 0.39 to 61.3 ± 0.63 ml. However, the effect of PEG was not significant in 0 to 96 h incubation, but the UPEG produced more gas in 0 to 3 and 3 to 6 h (P < 0.05). The gas production from the sample ground to pass through a 1-mm sieve was more for 3-mm sieve. This result indicated that the delay in gas production in treatment group of 1 to 3 mm is due to tannin, because the PEG significantly increased the amounts of gas production due to neutralizing negative effects of tannin on fermentation. Steingass (1983) has shown that the delay in the gas production is more pronounced with substrate containing unfermentable substrate. In this study, reduction in the size of sample from 3 to 1 mm did increase the amount of gas (P < 0.05). In the study of Steingass (1983), gas production from straw ground to pass 1 mm sieve was more than straw with 3 mm. However, Menke and Steingass (1988), have shown that a clear depression in gas production (calculated as ml/100 mg DM) could be observed if the amount of sample with the highest contents of CTs was increased from 200 to 400 mg DM, and 1 g PVP increased the amount of gas production. One of the possible reasons for this could be that tannins decreased the attachment of microbes to feed particles (Makkar et al., 1995). The exponential increase in gas production might be due to the fact that in addition to the microbially produced CO_2 gas, more CO_2 is being released from dissolved HCO_3 salts by volatile fatty acids (Menke and Steingass, 1988). The positive correlation with the PF indicates that the effect of tannin is more strongly reflected in the reduction of gas production than in the reduction of DMD. The PF values, which varied from 4.64 to 12.05 to values of 4.74 to 7.84 upon the addition of PEG, indicate the inhibitory effects of tannins on gas production. This suggests that the presence of tannin has a potentially beneficial effect on protein nutrition of the host animal by altering partitioning of nutrients towards higher microbial yield rather than short chain fatty acid. Ben et al. (2000), showed that sheep on tanniniferous diet (*Acacia foliage*) supplemented with PEG, exhibited higher microbial synthesis as compared to those fed diet without PEG. Previous works has shown that PEG-tannin complexes can lower the apparent and true digestibility's *in vitro* studies (Jones et al., 2000; Palmer and Jones, 2000). The results of this study suggest that the presence of tannins in raisin stalk is associated with a reduction in nutrients digestibility and *in vitro* fermentation. These deleterious effects of tannins can be neutralized by PEG-6000 supplementation.

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