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Effect of Polyethylene Glycol and Polyvinyl Pyrrolidon on i*n Vitro* Gas production of Pomegranate Seed

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ABSTRACT: The aim of this study was to determine the chemical composition including tannin content of pomegranate seed supplemented with different levels of tannin-binding agents (polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP)) and gas production characteristics using *in vitro* gas production technique. The treatments were PS (control), PS+150 mg PEG, PS+300 mg PEG, PS+150 mg PVP and PS+300 mg PVP. At the 48 h incubation times, the gas production volume of PS, PS+150 mg PEG, PS+300 mg PEG, PS+150 mg PVP and PS+300 mg PVP were 109.47, 129.71, 125.79, 125.22 and 99.99 ml/g DM, respectively, and treatments PS+150 mg PEG, PS+300 mg PEG and gas production of PS+150 mg PVP treatment was higher (P<0.05) than other treatments. This value for treatment of PS+300 mg PVP revealed to be lower than the others (P<0.05). Addition of PEG and PVP could overcome adverse effects of tannins on nutrient availability as indicated by gas production parameters. Addition of PEG and PVP (except 300 mg PVP level) inactivated effects of tannins and increased gas production in pomegranate seed.
Key Words: By-Product, *In Vitro* Gas Production, Polyethylene Glycol, Polyvinylpyrrolidon, Pomegranate Seed, Tannin.

INTRODUCTION

The developing countries experience serious shortages of animal feeds and fodders of the conventional type. In order to meet the projected high demand of livestock products, and to fulfill the future hopes of feeding the millions and safeguarding their food security, the better utilization of non-conventional feed resources which do not compete with human food is imperative. There is also a need to identify and introduce new and lesser known food and feed crops. An important class of non-conventional feeds is by-product feedstuffs which are obtained during harvesting or processing of a commodity in which human food or fiber is derived. The amount of by-product feedstuffs generally increases as the human population increases and economies grow (Besharati et al., 2008). Several factors have led to increase interest in by-product feedstuffs, such as pollution abatement and regulations, increasing costs of waste disposal, and changes in perception of the value of by-product feedstuffs as economical feed alternatives (Besharati et al., 2008).

Addition of pomegranate by-products in ruminant diets can improve the utilization of low-quality roughages mainly through the supply of protein to rumen microbes, but the presence of tannins in these byproducts prevents not only their optimal utilization but also that of the roughages and byproducts. Addition of a tannin-complexing agent, PVP, and PEG to tannin-rich diets is another attractive option to enhance the feeding value of such diets (Besharati and Taghizadeh, 2011).

For about 3 decades, it has been known that tannins bind to PVP and PEG. Polyvinylpyrrolidon and PEG are also considered to break already formed tannin-protein complexes, as their affinity for tannins is higher than for proteins. This property of these tannin-complexing agents, in particular of PEG of molecular weight 3500 or 4000, were exploited by various workers to alleviate the effects of tannins (Makkar, 2003). Addition of PEG results in the formation of PEG-tannin complexes which inactivates tannins. PVP and PEG of different molecular weights are available commercially. Systematic investigations were conducted on the binding efficiency of PVP (molecular weights: 10,000, 40,000, and 360,000) and PEG (molecular weight: 2000–35,000) in order to identify the most effective tannin-complexing agents (Makkar et al., 1995). The affinity of PVPs for tannins was lower than of PEGs. Furthermore, binding of insoluble PVP (PVPP) to tannins was lowest at pH = 7 and the binding with PEG 6000 was the same from pH = 4.7–7, except for quebracho tannins for which the binding increased as the pH approached 7. The binding with PEG 2000 decreased to a greater extent, as the pH reached near neutral, and for PEG 4000 this decrease was slightly lower. The PEGs were the most effective followed by PVPs and PVPP. The PEG 35,000 was the least effective amongst PEGs and the efficiency of other PEGs was similar. The PEG 6000 may be preferred for inactivation of tannins in feedstuffs as its binding to tannins was highest at near neutral pH values (Makkar, 2003). Addition of PEG to tannin-containing feeds increased *in vitro* gas and SCFA production and *in vitro* degradation of nitrogen. Therefore, there appears to be a potential for improving the utilization of tannin-containing feeds by the use of tannin binding agent such as PEG without altering the genetic pool of tannin-containing plants. Studies concluded that inclusion of energy sources with the aim of synchronizing nitrogen degradability and availability of energy increased the efficiency of microbial protein synthesis in the presence of PEG (Getachew et al., 2000). This approach can be used both by farmers and by the industry. Farmers can give PEG directly to animals through water, by mixing it with a small amount of concentrate, by spraying it on tannin rich feedstuffs or better still as a part of nutrient blocks. Industry can incorporate PEG in a pelleted diet composed of ingredients including tannin-rich byproduct(s) (Makkar et al., 1995).

There is a little information available regarding the nutritive value of pomegranate seed (PS) produced in Iran. The aim of this study was to determine the chemical composition including tannin content of pomegranate seed supplemented with different levels of tannin-binding agents PEG and PVP and gas production characteristics using *in vitro* gas production technique.

MATERIALS and METHODS

Pomegranate By-Product

Pomegranate seed (PS) was obtained from fruit juice manufacturing factory of Tabriz, Iran.

Chemical composition

Pomegranate seed dry matter (DM, method ID 934.01), ash (method ID 942.05), ether extract (EE, method ID 920.30) and crude protein (CP, method ID 984.13) were determined by procedures of AOAC (1999). The NDF and ADF concentrations were determined using the methods of Van Soest et al. (1991) without sodium sulphite. NDF was analyzed without amylase with ash included.

Total phenolics (TP) were measured using the Folin Ciocalteau method (Makkar, 2000). Total tannin (TT) was determined after adding insoluble polyvinylpyrrolidone and reacting with Folin Ciocalteau reagent (Makkar, 2000). Tannic acid was used as the standard to express the amount of TP and TT.

In vitro gas production trial

The dry matter degradability of each by-product was determined by *in vitro* fermentation with ruminal fluid obtained collected approximately 2 h after morning feeding from two cannulated sheep consuming 400 g alfalfa hay, 300 g barley and 300 g soybean meal. Ruminal fluid was immediately squeezed through four layers of cheesecloth and was transported to the laboratory in a sealed thermos. The resulting ruminal fluid was purged with deoxygenated CO_2 before use as the inoculum. Gas production was measured by Fedorak and Hurdey (1983) method. The treatments were PS (control), PS+150 mg PEG, PS+300 mg PEG, PS+150 mg PVP and PS+300 mg PVP. Approximately 300 mg of dried and ground (2mm) Pomegranate seed with (150 or 300 mg) and without PEG (6000) or PVP (25000) were weighed and placed into serum bottles. Each treatment was replicated three times. Buffered rumen fluid with McDougal buffer (20ml) was pipetted into each serum bottle (McDougall, 1948). The gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, and 48 h of incubation. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml per 1 g of DM.

Statistical analysis

Data obtained from *in vitro* gas production study was subjected to analysis of variance as a completely randomized design by the GLM procedure of SAS Institute Inc. (2002) and treatment means were compared by the Duncan test.

RESULTS and DISCUSSION

The chemical compositions of pomegranate seed are shown in Table 1. Crude protein, ADF, NDF, EE, ASH, TP and TT contents in pomegranate seed were 12.2%, 44.6%, 62.3%, 1.6%, 12.1%, 1.8% and 0.8%, respectively. Chemical compositions of pomegranate seeds in the current study were inconsistent with findings of Taher-Maddah et al. (2012). Feizi et al. (2005) reported that DM, OM, CP, crude fiber, and EE values of pomegranate seeds were 94.8, 96.8, 11.4, 38.9, and 1.0%, respectively. These differences in chemical composition of by-products may be due to a difference in cultivar, growing conditions, varieties, and different de-hulling process methods (Taher-Maddah et al., 2012).

Total gas production volume of treatments in incubation times (ml/g DM) are presented in Table 2. Addition of PEG or PVP to tannin-containing feeds increased *in vitro* gas production. At the early incubation times, the control treatment (treatment without PEG or PVP) maintained the lowest *in vitro* gas production volume within treatment ($P<0.05$). At the 6 h of incubation time, the treatment PS + 150 mg PVP had the highest gas production volume among treatment ($P<0.05$). After 6 h of incubation time, the treatments with PEG or PVP showed higher gas production compared to control treatment (treatment without PEG or PVP; $P<0.05$).

At the 48 h of incubation time, the gas production volume of PS, PS+150 mg PEG, PS+300 mg PEG, PS+150 mg PVP and PS+300 mg PVP were 109.47, 129.71, 125.79, 125.22 and 99.99 ml/g DM, respectively, and treatments PS+150 mg PEG, PS+300 mg PEG and PS+150 mg PVP resulted the highest gas production volume ($P<0.05$).

Table 1. Chemical composition of Pomegranate seed (% DM)

	Items							
	DM	CP	EE	NDF	ADF	ASH	TP	TT
Pomegranate seed	93.8	12.2	1.6	62.3	44.6	12.1	1.8	0.8

DM= Dry matter; CP= Crude protein; EE= Ether extract; ADF= Acid detergent fiber; NDF= Neutral detergent fiber; TP= Total phenol; TT= Total tannins.

Table 2. Total gas production volume (ml/g DM) in incubation times

Treatments	Incubation times (h)								
	2	4	6	8	12	16	24	36	48
PS	31.52[c]	33.45[c]	38.11[c]	57.42[c]	82.51	87.79	95.11[c]	106.50[b]	109.47[b]
PS + 150 mg PEG	34.63[b]	44.11[b]	50.43[b]	69.74[ab]	82.10	88.49	105.14[ab]	119.86[a]	129.71[a]
PS + 300 mg PEG	33.74[bc]	39.56[b]	48.87[b]	66.85[b]	81.66	91.05	106.47[ab]	119.41[a]	125.79[a]
PS + 150 mg PVP	37.74[a]	48.44[a]	61.08[a]	76.18[a]	84.32	90.94	108.14[a]	119.53[a]	125.22[a]
PS + 300 mg PVP	35.30[ab]	43.55[b]	53.09[b]	75.07[a]	83.88	88.05	97.82[cb]	98.08[c]	99.99[c]
SEM	0.82	1.52	2.12	1.98	2.59	3.38	2.67	1.76	1.50

The means within a column without common letter differ (p<0.05).

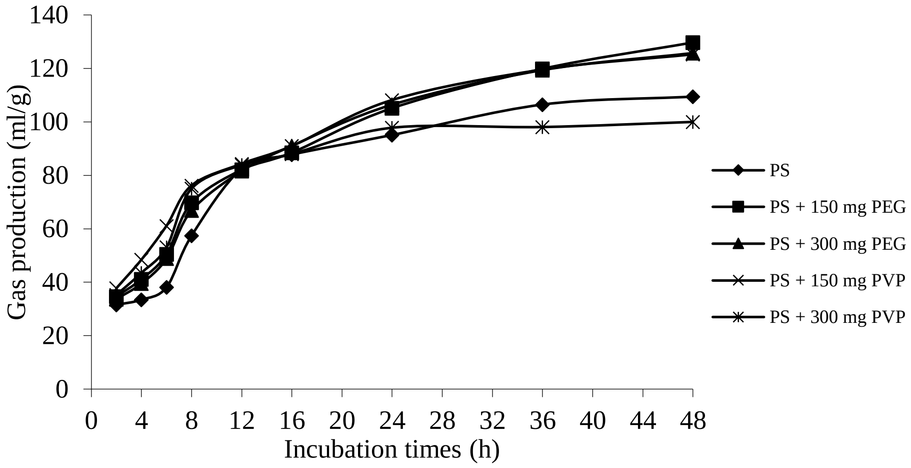

Figure 1. The effect of PEG and PVP on gas production of PS.

The treatment PS+300 mg PVP revealed the lowest gas production volume ($P<0.05$). The pattern of *in vitro* gas production of treatments were shown in Figures 1.

The PEG or PVP supplementation had a significant effect on *in vitro* gas production of PS. These results are in lined with the findings of Getachew et al. (2001), Getachew et al. (2002), Seresinhe and Iben (2003) and Singh et al. (2005). Tannins bind to protein and decrease accessibility of proteins to rumen microorganisms.

Tannins may form a less digestible complex with dietary proteins and may bind and inhibit the endogenous protein, such as digestive enzymes (Kumar and Singh, 1984). Tannin can adversely affect the microbial and enzyme activities (Singleton, 1981; Lohan, 1983; Barry and Duncan, 1984; Makkar et al., 1989). Hagerman et al. (1992) reported that tannins reduced CP digestibility. In another study, McNeill et al. (2000) showed that increasing condensed tannin in diet from 6 to 65 g/kg DM, decreased N digestibility from 0.805 to 0.378 and also increased excretory N in sheep feces from 4.3 to 9.7 g/d. Besharati and Taghizadeh (2009) showed that addition of dried grape by-product to basal decreased digestibility of CP ($P<0.05$), also increasing of dried grape by-product supplementation level had linear effect on CP digestibility of diets ($P<0.05$). The substantial reduction in N digestibility as a result of the presence of tannins was similar to that reported in sheep fed *Lotus pedunculatus* as a sole diet and when *Lotus pedunculatus* was fed with ryegrass (*Lolium perenne*) (Waghorn and Shelton, 1995), with and without polyethylene glycol (PEG). Polyethylene glycol, a non-nutritive synthetic polymer, has a high affinity to tannins and makes tannins inert by forming tannin PEG complexes (Makkar et al., 1995). Polyethylene glycol also can also liberate protein from the preformed tannin-protein complexes (Barry et al., 1986). 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. (1999) showed that the addition of PEG caused a significant and marked increase in the rate and extent of ammonia production in the rumen. Tannins also have effects on carbohydrates, particularly hemicellulose, cellulose, starch and pectins (Schofield et al., 2001).

Kamalak et al. (2007) reported that total and soluble condensed tannins, NDF and ADF were negatively correlated with estimated parameters of gas production. The results in our study are consistent with those of Feizi et al. (2005) who stated that pomegranate peel tannins had a negative effect on *in vitro* rumen fermentation. Tannins are considered to have both adverse and beneficial effects in ruminant animals. High concentrations of tannins may reduce intake, digestibility of protein and carbohydrates, and animal performance through their negative effect on palatability and digestion. By preventing bloat and increasing the flow of non-ammonia nitrogen and essential amino acids from the rumen, low and moderate (20-45 mg/g DM) concentrations of condensed tannins in the diet improved production efficiency in ruminants, without increasing feed intake (Shabtay et al., 2008). Recentely, there is an increasing interest of nutritionists in bioactive plant factors - phytofactors as natural feed additives, tannins and etc. that can modify the rumen fermentation processes (e.g., defaunation), improve the protein metabolism and, at the same time, reduce ammonia production and emission, and curb methane production and emission to the atmosphere. High diversity of bioactive phytofactors contained in many plant species has been identified as a potential factor affecting the above-mentioned processes (Szumacher-Strabel and Cieślak, 2010).

CONCLUSION

Addition of PEG and PVP could overcome adverse effects of tannins on nutrient availability as indicated by gas production parameters. Addition of PEG and PVP inactivated effects of tannins and increased gas production in pomegranate seed. However, there is not enough of information available about feasibility of using PEG and PVP in tannin-rich diets for ruminants. Prior to use of a large scale, PEG and PVP supplementation to improve the nutritive value of pomegranate yield by-products should be further analyzed in detail whether it is economically feasible due to their high.

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