Journal of Animal and Veterinary Advances 6 (9): 1135-1139, 2007
ISSN: 1680-5593

Effects of Polyethylene Glycol (PEG) Addition on Chemical Composition, Degradability and Digestibility of White Grape Pomace

[1]Rasoul Pirmohammadi, [2]Omid Hamidi and [3]Ali Mohsenpur Azari
[1]Department of Animal Science, Faculty of Agriculture, Urmia University, 165 Urmia, Iran
[2]Department of Animal Science, Islamic Azad University, Maragheh Branch, Maragheh, Iran
[3]W. Azerbaijan Agricultural and Natural Resources Research Center, Urmia, Iran

Abstract: The Grape Pomace (GP) is one of the agro-industrial by-products that can be used in ruminant feeding. But it has anti-nutritional factors such as tannins. An experiment was carried out to study the effect of Polyethylene Glycol (PEG) addition on chemical composition, degradability and digestibility of white grape pomace for ruminants. Inclusion rate of PEG to GP was done at 4 levels. The nylon bag technique was used to determine the rate of degradability of DM with 3 rumen-fistulated Azeri buffaloes. Dry Matter and Organic Matter Digestibility (DMD and OMD) were determined using *in vitro* methods. Results showed that NDF content of WGP increased with PEG inclusion. Crude Protein (CP) concentration of untreated WGP (No PEG) was 13.25% and for WGP1 (15.60 g kg^{-1} PEG), WGP2 (31.21 g kg^{-1} PEG) and WGP3 (46.80g kg^{-1}PEG) were 11.41, 10.80 and 10.56%, respectively. TEPH (total extractable phenol) concentration of WGP (No PEG), WGP1 (15.60 g kg^{-1} PEG), WGP2 (31.21 g kg^{-1} PEG) and WGP3 (46.80g kg^{-1}PEG) were 2.36, 1.43, 1.65 and 1.49%, respectively. These observations may show the positive effects of PEG addition to increase degradability value of WGP. According to results of this study it may conclude that inclusion of PEG to WPG can make a progress in degradability and digestibility values and the optimum effect was obtained at WGP2 (31.21 PEG g kg^{-1} DM of WPG) inclusion level.

Key words: Grape pomace, PEG, degradability, digestibility, chemical composition, CP

INTRODUCTION

The Grape Pomace (GP) is one of the agro-industrial by-products that can be used in ruminant feeding. But it has anti-nutritional factors such as tannins. Tannins are poly phenolic substances with various molecular weights and a variable complexity (Makkar, 2003). Tannins have ability to bind with other nutrients particularly proteins (Makkar, 2003) and fiber (Makkar, 2003; Ben Salem, 1999). Tannins tentatively classified into two classes: Hydrolysable and condensed tannins (Makkar, 2003). The condensed tannin-protein and condensed tannin-NDF complexes in feedstuffs are important because these complexes can lead to a decrease in nutritional value of the feeds (Makkar, 2003). *In vitro* digestibility and in situ degradation studies showed positive responses to incubation of the tannin-containing plant samples with tannin-binding agents in comparison to non-treated samples (Silanikove *et al*., 2001).

Various studies aimed at detoxification (by inactivation or removal of tannins) and increasing the nutritional value of some tanniniferous feeds like oak leaves (Makkar, 2003). Polyethylene Glycol (PEG) is known to preferentially bind with condensed tannins and thus prevent the formation of potentially indigestible tannin-protein complexes (Jones and Palmer, 2000). PEG was added to tanniniferous feeds at various extent and rates (Ben Salem, 1999).

Inclusion of PEG has led to variable results (Palmer and Jones, 2000b; Ben Salem, 1999). Such findings may be attributable to plant species, animal species, tannin levels and possibly tannin structure (Ben Salem, 1999).

This study was conducted to determine the effects of Polyethylene Glycol (PEG) addition on chemical composition, degradability and digestibility of white grape pomace for ruminants.

MATERIALS AND METHODS

Samples: The White Grape pomace was cached from main Juice factories in Urmia city. Inclusion rate of PEG to GP was done according to suggestions of (Palmer and Jones,

Corresponding Author: Rasoul Pirmohammadi, Department of Animal Science, Faculty of Agriculture, Urmia University, 165 Urmia, Iran

Table 1: Addition of PEG (6000) to White Grape pomace at different levels

Level of addition (g kg^{-1} DM of GP)	White Grape Pomace
Level 1	(No PEG)
Level 2	15.60
Level 3	31.21
Level 4	46.80

Table 2: The chemical composition of experimental grape pomaces

Chemical composition (%DM)	WGP No PEG	WGP1	WGP2	WGP3
DM	39.10	37.50	36.40	35.71
ASH	7.20	7.11	6.44	7.02
CP	13.25	11.41	10.80	10.56
NDF	50.40	53.03	53.11	53.24
TEPH	2.36	1.43	1.65	1.49
TET	1.86	0.90	1.19	1.02

DM = Dry Matter, CP = Crude Protein, NDF= Neutral Detergent Fiber, TEPH= Total Extractable Phenol, TET= Total Extractable Tannin, WGP = White Grape Pomace, WGP1= adding PEG to White Grape Pomace at level 1, WGP2= adding PEG to White Grape Pomace at level 2, WGP3= adding PEG to White Grape Pomace at level 3

2000b; Ben Salem, 1999), at 4 levels (Table 1). Each 5 g PEG dissolved in 10 mL water and then sprayed to GP samples at various levels (Bhatta *et al*., 2002).

Chemical analyses: The sample ingredients were analyzed using methods recommended by AOAC (1990) (Table 2). Dry matter was determined by drying the whole sample in an oven at 65°C until a constant weight was achieved (AOAC, 1990). Determinations of CP were conducted using the Kjeldahl method in an automated Kjelfoss apparatus (Foss Electric, Copenhagen Denmark). Neutral Detergent Fiber (NDF) was determined according to Van Soest *et al*. (1991). Total Phenolics (TP) were measured using the Folin Ciocalteau method (Makkar, 2000). Total Tannin (TT) was determined after adding insoluble Polyvinyl Polypyrrolidone (PVPP) and reacting with Folin Ciocalteau reagent (Makkar, 2000).

***In situ* study:** The nylon bag technique was used to determine the rate of degradability of DM with 3 rumen-fistulated Azeri buffaloes (AFRC, 1992). All of the dried samples ground and milled through a 2 mm sieve. Buffalos were fed with a maintenance extent diet. Then 5g of each sample put in the nylon bags (21×0 cm with a pore size of 45μm) and incubated in the rumen for 0, 3, 6, 12, 24, 48 and 96 h. In each buffalo one bag was used for each time interval. After withdrawing the bags in incubation times from the rumen, they were washed with cold water by a washing machine for 1 h. When all the bags had taken from the rumen, they were dried for 48 h at 70°C in oven.

For each bag, the residue was analyzed for DM and protein. The degradability at each time interval was calculated by taking the mean value obtained from the 3 bags. The percentage of degradability (Y) of DM and protein at time (t) was obtained from an exponential curve of the type: $Y = a + b (1 - e^{(-ct)})$.

This was fitted to the exponential data by iterative regression analysis (Ørskov and McDonald, 1979). In the equation, 'e' is the base of natural logarithms, the constant 'a' represents soluble and very rapidly degradable component and 'b' represents the insoluble but potentially degradable component, which degrades at a constant fractional rate © per unit time. The effective degradability of DM and protein in each feedstuff was then estimated (Ørskov and McDonald, 1979) by the following equation:

$$\text{Effective degradability (\%)} = \frac{a + b}{c + k}$$

In this equation, k refers to the fractional outflow rate of small particles from the rumen. A value of 0.05 fraction h^{-1} for k was used.

***In vitro* study:** Dry Matter and Organic Matter Digestibility (DMD and OMD) were determined using (Tilley and Terry, 1963) method as follows:

Preparing of samples: Primarily, samples milled through a 2 mm sieve. Then, from each sample was put 2 g and spilled into 100 mL dishes.

Buffer supply: Buffer was contained 9.8 g Sodium bicarbonate, 9.35 g Mono hydrogen sodium phosphate, 0.57 g Potassium chloride, 0.47 g Sodium chloride and 0.12 g Magnesium sulfate, solve in distilled water. Then, before starting of anaerobic digestion, 1 mL Calcium chloride 4% solution, added to buffer (per 1 liter of buffer) and solution reached to 1 L. Buffer pH set in 6.9-7 ranges with entering CO_2 gas for 10-15 min. Forty milliliter of solution used for each sample.

Rumen fluid supply: Rumen fluid prepared by rumen fistulated Azeri buffaloes that were fed with a maintenance diet. Rumen fluid kept in anaerobic conditions in 39 °C water.

Anaerobic digestion: Filtered rumen fluid mixed with the buffer 4:1 ratio then co2 gas entered for getting anaerobic culture for 4-5 min in 39 °C. Fifty milliliter from this mixture spilled on 100 mL flasks that contained GP sample. Then let the flasks to be incubated in 39°C for 48 h (flasks was shake each 8h interval).

Acidic pepsin digestion: After anaerobic digestion, 6 mL of Hydrochloride 20% solution was added to all of the

flasks in 3 steps respectively (4, 1, 1 mL). Then 2 mL of Pepsin 5 % solution was added to the flasks then they incubated in 39°C for 46h (flasks was shake each 8 h interval).

Separating of no digest content: Separating of no digest content was done using ash less Watt man strainer paper number 41.

Determination of residual digestion and ash: Residual material weight determined by drying of separated material using strainer paper in 72°C for 48h. Furthermore, ash weight of residual digestion determined with 550 °C oven for 3.5 h.

Determination DM and OM digestion: Determination of digestibility was used with the equations suggested by (Tilley and Terry, 1963).

Statistical analysis: Data obtained from this study were subjected to ANOVA statistical analysis and mean values were compared with Duncan test.

RESULTS AND DISUSSION

Chemical analysis: Neutral Detergent Fiber (NDF) content of untreated WGP (No PEG) was 50.40 % and for WGP1 (15.60 g kg^{-1} PEG), WGP2 (31.21 g kg^{-1} PEG) and WGP3 (46.80g kg^{-1}PEG) were 53.03, 53.11 and 53.24 %, respectively (Table 2). These results showed that NDF content of WGP increased with PEG inclusion. This may be due to formation of tannin-PEG complexes, which is not soluble in Neutral detergent solutions and consequently shows NDF concentration more that true one (Makkar, 2003).

Crude Protein (CP) concentration of untreated WGP (No PEG) was 13.25% and for WGP1 (15.60 g kg^{-1} PEG), WGP2 (31.21 g kg^{-1} PEG) and WGP3 (46.80g kg^{-1}PEG) were 11.41, 10.80 and 10.56%, respectively (Table 2). This outcome showed that CP content of WGP decreased with increasing level of PEG inclusion. This may partly attribute to diluting effect of PEG inclusion to WGP. As described above in materials and methods, Each 5 gr PEG dissolved in 10 mL water and then sprayed to GP samples at various levels.

TEPH (total extractable phenol) concentration of untreated WGP (No PEG), WGP1 (15.60 g kg^{-1} PEG), WGP2 (31.21 g kg^{-1} PEG) and WGP3 (46.80g kg^{-1}PEG) were 2.36, 1.43, 1.65 and 1.49%, respectively (Table 2). This finding showed that TEPH content of WGP decreased with increasing level of PEG addition. Furthermore, similar trend has been showed with TET concentration of the treatments. It seems that added PEG makes strong hydrogenous complexes with tannins (Ben Salem, 1999) and these complexes can not be disconnected with Folin Ciocalteau reagent (Makkar, 2000) we used in this study to determine tannins, then decreasing of phenolic compounds with addition of PEG is expectable. Such findings have harmony with other assessments (Ben Salem, 1999).

***In situ* degradability:** Dry matter degradability of the WGP samples was shown in (Table 3). Soluble fraction (a) of DM degradability of untreated WGP (No PEG) was 25.39 % and for WGP1 (15.60 g kg^{-1} PEG), WGP2 (31.21 g kg^{-1} PEG) and WGP3 (46.80g kg^{-1}PEG) were 29.01, 29.26 and 30.69 %, respectively (Table 3). These data indicated that *a* fraction of DM degradability of WGP increased with PEG inclusion ($p<0.05$). This may be due to formation of tannin-PEG complexes, which may release soluble components of WPG and consequently increase the degradation of soluble fraction. Our results are in agreement with (Ahn *et al*., 1989) who worked on quality assessment of tropical browse legumes tannin content protein degradation. But some reports showed that PEG inclusion might have variable differences based on plant species, animal species, tannin levels and possibly tannin structure (Ben Salem, 1999).

The insoluble but fermentable component (*b* fraction) of WGP (No PEG), WGP1 (15.60 g kg^{-1} PEG), WGP2 (31.21 g kg^{-1} PEG) and WGP3 (46.80g kg^{-1}PEG) were 25.63, 20.79, 20.69 and 18.98%, respectively (Table 3). This outcome showed that (b fraction) of WGP decreased with increasing level of PEG addition ($p<0.05$). The decrease of b is obviously due to increase of *a* fraction, which was discussed above.

Rate of degradation (c fraction) were not significant among WGP samples ($p>0.05$). However, DM Effective Degradability (ED 0.05) of WGP.1 (37.24), WGP.2 (39.17) and WGP.3 (38.60), were significantly ($p<0.05$) higher than that of WGP (No PEG), (35.97)% and WGP.2 had the highest ED (0.05) value among the samples ($p<0.05$). These observations may show the positive effects of PEG addition to increase degradability value of WGP and confirm some other in situ rumen nylon bag reports (Ahn *et al*., 1989).

***In vitro* digestibility:** digestibility values of WGP samples were given in (Table 4). The IVDMD (*in vitro* dry matter digestibility) and IVOMD (*in vitro* organic matter digestibility) and IVDOMD (*in vitro* dry organic matter digestibility) values for WGP (No PEG) were 34.50, 33.57,

Table 3: Mean differences of Degradation Values (DM) for WGP samples (% DM)

	Items				
Treatments	a	b	a + b	c	ED (0.05)
WGP No PEG	25.39[a]	25.63[a]	51.01[a]	0.037[a]	35.97 a
WGP1	29.01[b]	20.79[b]	49.79[a]	0.034[a]	37.24ab
WGP2	29.26[b]	20.69[b]	49.95[a]	0.050[a]	39.17 c
WGP3	30.69[b]	18.98[b]	49.66[a]	0.038[a]	38.60bc
P value	0.05	0.05	ns	ns	0.05

a = rapidly degraded fraction (%); b = slowly degraded fraction (%); c = rate of degradation (fraction/h); are constants in the exponential equation [$p = a + b\ (1 - e^{-ct})$]; ED (%) = effective degradability (out flow rate) Means in the same row with difference superscripts (a, b, c) letters are significantly difference, WGP= White Grape pomace, WGP1= adding PEG to White Grape Pomace at level 1, WGP2= adding PEG to White Grape Pomace at level 2, WGP3= adding PEG to White Grape Pomace at level 3

Table 4: The digestibility values of WGP with various levels of PEG

	Treatments				
Items	WGP No PEG	WGP1	WGP2	WGP3	P value
IVDMD (%)	34.50a	38.00b	38.00b	37.00ab	0.05
IVOMD (%)	33.57a	38.21b	38.68b	37.47ab	0.05
IVDOMD (%)	31.16a	35.50b	36.20b	34.84ab	0.05

IVDMD = *in vitro* Dry Matter Digestibility, IVOMD= *in vitro* Organic Matter Digestibility, IVDOMD= *in vitro* Dry Organic Matter Digestibility, Means in the same column with difference superscripts (a, b) letters are significantly difference WGP= White Grape pomace, WGP1= adding PEG to White Grape Pomace at level 1, WGP2= adding PEG to White Grape Pomace at level 2, WGP3= adding PEG to White Grape Pomace at level 3

and 31.16%, respectively and these values for WGP.1 were 38.00, 38.21 and 35.50%, respectively and for WGP.2 were 38.00, 38.68 and 36.20%, in that order and for WGP.3 were 37.00, 37.47 and 34.84 (% DM), correspondingly. Each 3 values for WGP (No PEG) were significantly ($p<0.05$) lower than that of the PEG treated samples, but there were no significant differences within the PEG treatments. Similar results reported by Palmer and Jones (2000a) also they lay emphasis on that the IVDMD value in *in vitro* studies may be lower than the true IVDMD value and they were calculated CIVDMD (corrected *in vitro* dry matter digestibility) with ^{14}C-labelled PEG.

CONCLUSION

According to results of this study it may conclude that inclusion of PEG to WPG can make a progress in degradability and digestibility values and the optimum effect was obtained at WGP2 (31.21 PEG g kg^{-1} DM of WGP) inclusion level. Because of more complicated behavior of tannins in different feedstuffs, more investigation should be done with GP and other tanniniferrous compounds with PEG and other chemicals.

ACKNOWLEDGEMENT

The authors thank W. Azerbaijan Agricultural and Natural Resources Research Center for financial support, Mr.G, Manafi Azar and B, Afshar Hamidi for their assistance and Dr. A. Safamehr for his scientific help.

REFERENCES

Agricultural and Food Research Council (AFRC), 1992. Nutrient Requirements of Ruminant Animals: Protein. Technical Committee on Responses to Nutrients, Report No. 10. Nutr. Abst. Rev. Series B, 62: 787-835.

Ahn, J.H., B.M. Robertson, R. Elliot, R.C. Gutteridge, C.V. Ford. 1989. Quality assessment of tropical browse legumes tannin content and protein degradation. J. Anim. Feed Sci. Tech., 27: 147-156.

Association of Official Analytical Chemists (AOAC), 1990. Official Methods of Analysis. (15th Edn.), Association of Official Analytical Chemists, Arlington, VA, USA, Vol. II.

Bhatta, R. *et al*., 2002. Effect of polyethylene glycol-6000 on nutrient intake, digestion and growth of kids browsing *Prosopis cineraria*. Anim. Feed Sci. Tech., 101: 45-54.

Ben Salem, H. *et al*., 1999. Intake, digestibility, urinary excretion of Purine derivatives and Growth by sheep given fresh, air-dried or polyethylene glycol-treated foliage of Acacia Cyanophylla Lindl. Anim. Feed Sci. Tech., 78: 297-311.

Palmer, B. and R.J. Jones, 2000a. *In vitro* digestion studies using 14C-labelled Polyethylene Glycol (PEG): The effect of sample pretreatment on dry matter and nitrogen digestibility as well as PEG binding of Callindra calothyrsus. J. Anim. Feed Sci. Tech., 86: 149-155.

Palmer, B., R.J. Jones, 2000b. The effect of PEG addition *in vitro* on dry matter and nitrogen digestibility of Callindra calothyrsus and Leucaena leucocephala leaf. J. Anim. Feed. Sci., Technol., 85: 259-268.

Makkar, H.P.S., 2000. Quantification of tannins in tree Foliage. A Laboratory Manual for The FAO/IAEA Co-ordinate Research Project on Use of Nuclear and Related techniques to Develop simple Tannin Assays for Predicting and Improving the safety and Efficiency of Feeding Ruminants on Tanninferous Tree Foliage. Joint FAO/IAEA, FAO/IAEA of Nuclear Techniques in Food and Agriculture. Animal Production and Health Sub-Programme, FAO/IAEA Working Document. IAEA, Vienna, Austria.

Makkar, H.P.S., 2003. Effects and fate of tannins and strategies to overcome detrimental effects of feeding tannin rich-feed. Small Rumin. Res. (A Rev.), 49: 241-250.

Silanikove, N., A. Perevolotsky and F.D. Proveza, 2001. Use of tannin-binding chemicals to assay for tannins and their negative postingestive effects in ruminants. J. Anim., Feed Sci., Tech., 91: 69-81.

Ørskov, E.R. and P. McDonald, 1979. The estimation of protein digestibility in the rumen from incubation measurements weighed according to rate of passage. J. Agric. Sci., Cambridge, 92: 499-503.

Jones, R.J. and B. Palmer, 2000. *in vitro* digestion studies using 14C-labelled Polyethylene Glycol (PEG) 4000: comparison of six tanniniferous shrub legumes and the grass panicum maximum. J. Anim. Feed Sci. Tech., 85: 215-221.

Tilley, J.M.A and R.A. Terry, 1963. A two-stage technique for the *in vitro* digestion of forage crops. J. Br. Grassland Soc., 18: 104-111.

Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods of dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation of animal nutrition. J. Dairy Sci., 74: 3583-3597.