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Influence of Drying and Ensiling Pistachio By-Products with Urea and Molasses on Their Chemical Composition, Tannin Content and Rumen Degradability Parameters

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Abstract: Chemical composition and degradability of Pistachio By-products (PB) were studied in 3 experiments. In the first experiment, PB were ensiled with urea (0 and 0.15%) and molasses (0, 1.5, 3.0 and 4.5%). Total phenols and tannins content of PB silages were reduced significantly ($p<0.05$) following Urea application. Their reduction rates were 60 and 80%, respectively. DM content of the treated PB silage with 4.5% molasses increased and significant ($p<0.05$) reduction was observed in phenols and tannin contents. In the second experiment, ruminal degradability coefficients were determined. The treatments were; dried PB (T_1), PB silage without urea and molasses (T_2) and PB silage with 0.15% molasses (T_3). The "a" fraction of DM in T_2 (0.379) was lower than T_1 and T_3. The "c" coefficient for DM in T_1 (0.025) was higher than T_2 and T_3. The "b" coefficient of CP, in T_3 (0.339) were lower than T_1 and T_2. The "c" coefficient of CP in T_2 (0.020) was higher than T_1 and T_3. In the third experiment, rumen and intestinal disappearances of DM and CP were studied. Ruminal disappearance and total digestibility of DM, in T_2 (46.28 and 54.70%) were lower than those for T_1 and T_3. Ruminal disappearance of CP differed significantly between the treatments. The intestinal disappearance of CP in T_1 (18.5%) was significantly higher than that for T_3 (13.6%). The results showed the dried PB had higher degradability and digestibility in comparison with the ensiled PB. More *in vivo* practical researches are required at farm levels.

Key words: Pistachio by-products, tannins, phenolic compounds, silage, degradability

INTRODUCTION

Low rainfall and availability of water resources for agricultural consumption are the main obstacles to Iranian farmers. Limited forages for grazing ruminants and feed shortage for the huge available animal numbers with low-productivity are the other well-known characteristics of the country animal industry (Statistical Center of Iran, 2007).

Water shortage as well as dry climatical conditions had increased costs of animal products in Iran similar to many other countries. Therefore, it is logical to be accepted that many attempts be made to utilized agricultural and agro-industrial by-products or residues obtained after crop harvesting or processing of fruit, vegetables and nuts such as Pistachio By-products (PB) for overcoming the noted problem.

Iran is the largest pistachio (nuts) producer in the world. About 58% of the total pistachio dry nuts are produced in Iran. Most of the pistachio farms (82%) are located in Kerman province (Vahmani *et al*., 2006; Shakeri *et al*., 2004) with a typical dry climatical conditions. Soon after pistachio harvesting the nuts must be de-hulled and their moisture content reduced to less than 10% what is called pistachio by-products weigh 1.5 time of the de-hulled dry nuts and comprise of 64.5% soft external hull, 25% twinges, 10% leaves, 0.5% kernel and woody shell (Vahmani *et al*., 2006; Bagheripour *et al*., 2008). Every year up to 400 tons PB is produced in Iran. This by-product can be deteriorated in open fields because of its high content of moisture and nutrients. Thus, it can be an environmental Pollution threat (Seied *et al*., 2003; Vahmani *et al*., 2006). Therefore, PB utilization as animal feed not only lowers feed shortage in the area but also reduces the risk of environmental pollution.

For the first time, Labavitch *et al*. (1982) reported that Crude Protein (CP), fiber and fat contents of PB are similar to the almond by-products. These workers also, reported that phenolic compounds of PB were 5-7 times more than almond by-products. The feeding value of PB varies largely depending on variations in pistachio cultivars,

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kernel maturity growing, harvesting, drying and de-hulling processes. For example, CP content of PB reported by Forough and Fazaeli (2005) was 9.2% while, the level of this nutrient for pistachio hulls measured by Vahmani and Naserian (2003) was 21.3%.

Pistachio by-products contain the high level of phenolic compounds and tannins, which can affect their nutrients utilization by animals (Reed, 1995). Seied *et al.* (2003) reported that amount of total phenolic compounds and tannins in PB were 15.6 and 10.2%, respectively. Bagheripour *et al.* (2008) stated that total phenols and total tannins as tannic acid equivalents contents of the sun-dried PB were 15.2 and 9.0%, respectively.

Pistachio by-products are produced in a short period of time and may not be utilized or stored in raw form with low dry matter content (<30%).

Very limited methods of raw PB preservation and tannins or phenols deactivation have been demonstrated (Makkar, 2003; Bapheripour *et al.*, 2008). It is therefore, the objectives of this experiment to test the easily applicable cost-effective methods of PB storage as well as reduce the adverse effects of tannins and phenols. This study was also, undertaken to investigate the feeding value of PB in case of ruminal degradability measures and digestion in the rumen and small intestine.

MATERIALS AND METHODS

Ensiling and detanninificantion: Fresh pistachio by-products containing soft hulls, twigs, leaves little amount of hard shells and green kernels obtained from de-hulling factories in Fizabad (Khorasan Razavi Province, Iran). Thirty-six, 5 kg samples were taken after complete mixing. Thirty-two samples were treated with urea and molasses in a completely randomized design with a factorial arrangement of 2×4. Two levels of urea, 0 and 0.15% and 4 levels of molasses, 0.0, 1.5, 3.0 and 4.5% (DM basis) were added to fresh PB and ensiled into double plastic bags for 40 days at room temperature. Each treatment was replicated 4 times (4 bags). Four, 5 kg samples were dried under sun light by spreading PB on concrete surface for 72 h.

At the end of ensiling period bags were opened and the representative samples taken for subsequent analyses. DM content was determined by drying the samples in an oven at 60°C for 48 h (AOAC, 1990). The dried samples were ground to pass a 2 mm sieve and their CP content was measured by kjeltec auto (1030 Analyzer Tecator), ADF and NDF by Van Soest *et al.* (1991) method. Total contents of phenols and tannins were determined according to the Folin and Ciocalteau (Makkar, 2000) procedure. pH of samples was determined by the following method. Fifty grams sample was taken from each bag (replicate) and 450 mL de-ionized distilled water added and mixed completely with electrical mixture. The pH of extracted liquid was determined by Metrohm 691 pH meter. Ammonia-N content of this extracted liquid was also measured by kjeltec Auto system.

Data on ensiling experiment were subjected to analysis procedure of SAS (2001) based on the following statistical model:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk}$$

Where:

- Y_{ijk} = The measured parameter for the ijk treatment,
- μ = The overall mean
- A_i = The ith effect of urea
- B_j = The effect of molasses on the observed parameters,
- Ab_{jk} = The interaction of urea and molasses
- e_{ijk} = The standard error term common for all observations

***In sacco* measurements:** The nylon-bag technique (Vanzant *et al.*, 1988) was used to measure the rumen degradability of the sun-dried and ensiled PB without additive and with 0.15% urea and 1.5% molasses (Orskov *et al.*, 1980). Four Holestin steers with the average initial live weight of 450±10 kg fitted with the permanent ruminal fistula in individual pens were used. The steers were offered a diet containing 50:50 forage to concentrate in 2 equal portions daily (0700 and 19.00).

Samples of about 3 g were weighed into polyester nylon bags with pore size of 44 μm and dimensions of 17×10 cm and incubated in the rumen for periods of 0, 2, 4, 8, 16, 24, 48, 72 and 96 h. After removal the bags were thoroughly washed under tap water and were dried to constant weight at 60°C.

The rapidly soluble materials were estimated by washing the bags containing samples after soaking in water without incubation in the rumen. The DM and CP degradation data were fitted to the following exponential equation (Orskov *et al.*, 1980):

$$P = a + b(1 - e^{-ct})$$

Where:

- P = Potential degradability or degradation of DM and CP at time t
- a = The rapidly soluble fraction
- b = The potentially degradable DM or CP
- c = The constant rate of degradation of b parameter (percentage h^{-1})

The obtained data from degradability measurements were analyzed by the Fig P software (Biosoft Corporation Durham, NC USA).

Degradability and digestibility measurements by the mobile nylon-bag technique: Four ruminally-fistulated steers (same as previously used animals) and 4 deodenal-canollated steers fed and managed as noted for in sacco experiment were used for this study in individual pens. The treatments were those noted for the previous experiment; sun-dried PB, ensiled PB without supplementation and ensiled PB supplemented by 0.15 urea and 1.5% molasses.

The mobile nylon bags utilized in this experiment (Van Hatalo *et al*., 1995; Bechers *et al*., 1996) had a pore size of 50 μm and 3×6 cm dimensions. Ground samples (Passed through 2 mm sieve) of about 1.2 g were weighed into the bags and sealed with the water proof paste. DM and CP degradabilities of the samples were determined by incubation the bags into the rumen for 12 h. These measurements were replicated 12 time (12 bags for each treatment).

DM and CP degradabilities of PB samples in the intestine and whole digestive tract were measured by incubation 16 bags in the rumen and duodenum for 12 h. After rumen incubation the bags were released into the duodenum through the canola and collected through feces. These mobile hags were washed, dried and analyzed as noted for the *in sacco* experiment. Data on these measurements were subjected to the SAS (2001) analyses procedure based on the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = Amount of each observation
μ = The general mean
T_i = The treatment effect
e_{ij} = The standard error term

RESULTS AND DISCUSSION

Table 1 shows that urea addition has had no effect on DM content of the ensiled PB. Whereas, molasses supplementation at levels >3% led to higher DM content ($p<0.05$) in comparison with the un-treated ensiled PB. High DM content of molasses had been the main reseon for this increment as reported by other workers (Umana *et al*., 1991). Urea supplementation also increased pH of the experimental silages probably due to ammonia formation as it had been reported elsewhere (Henderson, 1993). In contrast silages with molasses had significantly ($p<0.05$) lower pH value in comparison with the untreated silage. This fact was also imaginable due to high amount of soluble carbohydrate in molasses. However, low pH as reported in Table 1 is an appropriate indicator of a suitable silage (Henderson, 1993). McDonald *et al.* (1991) concluded that at lest 10% soluble sugars are required for obtaining good result from ensiling forages. Most of the workers have reported that soluble of PB is <9%, therefore, molasses addition can improve the quality of PB silages.

Urea addition resulted to higher content of CP in the ensiled PB but at the same time NH_3-N content of these silages increased. Although, molasses supplementation did not changed the CP content of the ensiled PB, but reduced significantly ($p<0.05$) the level of NH_3-N in these silages hence, CP content of PB can be increased with supplementation with urea in a suitable carrier as molasses. Such positive effects of this mixture (urea + molasses) had been frequently reported (Henderson, 1993; McDonald *et al*., 1991).

Total phenols and Tannins contents of the sun-dried PB in this study were 7.75 and 4.54%, respectively. Level of these compounds in ensiled PB (Table 1 and Fig. 1) Showed that ensiling process has reduced their contents significantly ($p<0.01$). Minimum reduction observed in PB silage without any additive. Reduction rate for phenols

Table 1: Chemical composition of ensiled PB with or without urea and molasses

	Urea level (DM%)			Molasses level (DM%)				
Items	0	0.15	SEM	0	1.5	3.0	4.5	SEM
DM (%)	29.50	29.60	0.13	27.7^{a}	29.5^{ab}	30.2^{b}	30.9^{b}	0.96
pH	4.74^{a}	5.19^{b}	0.05	4.75^{a}	4.22^{b}	4.05^{b}	3.9^{b}	0.08
NH_3-N (mg dL^{-1})	5.90^{a}	10.10^{b}	0.17	10.22^{a}	7.54^{b}	7.28^{b}	6.95^{b}	0.35
CP (%)	11.72^{a}	14.37^{b}	0.10	12.89	13.15	12.88	13.25	0.36
NDF (%)	34.84	34.36	0.34	35.35	34.66	34.31	34.07	0.92
ADF (%)	20.83	20.45	0.27	20.87	20.57	20.63	20.50	0.58
Total phenols (%)	5.64^{a}	4.55^{b}	0.08	4.47^{a}	5.63^{b}	5.51^{b}	4.51^{a}	0.15
Total tannins (%)	1.92^{a}	1.34^{b}	0.04	1.44^{a}	1.90^{a}	1.78^{b}	1.41^{a}	0.03

^{a,b}Means in row with different superscript letters are different *p = 0.05

and Tannins in this silage was 20 and 50% in comparison with dried PB. The maximum reduction was detected in PB silage treated with 0.15% Urea.

The reduction rates for phenols and tannins were 60 and 80%, respectively. Very limited reports are available on the effects of anaerobic ensiling process on phenols and tannins contents of PB. Most of the experiments have been done on tannin deactivation in sorghum (Mitaru *et al*., 1984; Reichert *et al*., 1980). Recently, Bapheripour *et al*. (2008) demonstrated that condensed and hydrolysable tannins and protein-precipitable phenols decreased following ensiling for 30 or 60 days. It was concluded from the tannin or phenol deactivation studies a positive correlation is exist between the water content of the silage and reduction rate of tannins or phenols. Therefore, the appropriate content of moisture in PB must be regarded as the key issue for producing a good quality silage from PB.

Urea addition was quite effective in reducing total phenols and tannins (Table 1 and Fig. 1). Probably, due to their polymerization as noted by Makkar (2000), Makkar and Singh (1993) in mature oak. They mixed fresh oak leaves with 4% urea and ensiled at moisture level of 55% for 10 days. The reduction rate for total phonols and the condensed tannins were 88 and 100%.

Degradability coefficients (Table 2) showed that "a" content of the control PB was lower than its level in the dried or ensiled PB with urea or molasses. Whereas, its "b" level was higher than the noted PB. The "c" constant rate for the dried PB was higher than the values reported for other PB forms in this study (Table 2). Probably, the main determintal effective compounds in this contest were tannins or phenols as emphasized by other researchers (Seied, 2003).

Although, amount of phenols and tannins in the control PB was lower than their levels in dried PB, but its DM degradability did not increased. Thus, it can be proposed that some other factors rather than phenols or tannins are involved in these measurements and more detailed studies are required.

The measured values for CP degradability (Table 2) indicated there was a negative correlation between the tannins content of PB and its CP degradability. These results are in agreement with the findings of Seied (2003) who reported with increasing level of PB in the goat diet, potential CP degradability reduced significantly ($p<0.05$). He emphasized that the condensed tannins were responsible for this reduction.

DM disappearance of the control PB (ensiled without additives) was significantly ($p<0.05$) lower than the dried or treated PB with urea and molasses (Table 3). However, intestinal DM disappearance did not differ between treatments. Khafipour (2001) obtained the similar results for alfalfa in dry or ensiled forms.

DM disappearance of the ensiled PB (without additives) was also significantly ($p<0.05$) lower than the obtained values for the dried or ensiled PB with urea and molasses (Table 3). Forough (1997), Forough and Fazaeli (2005) stated apparent DM digestibility of ensiled PB was higher than its dry form. He concluded tannins deactivation in the ensiling process was probably the main reason for this difference. In the present study although, tannin content of the ensiled PB was reduced but its digestibility did not increased and even decreased in some degrees. Therefore, some other compounds rather than tannins may be involved.

CP disappearance of PB samples in the rumen differed significantly ($p<0.05$). The maximum and minimum values were for ensiled PB with additives and ensiled PB Without additive (Table 3). Higher ruminal CP disappearance in this study was probably due to higher content of soluble-N obtained from urea addition (McDonald *et al*., 1991).

Intestinal CP disappearance of the dried PB was significantly ($p<0.05$) higher than ensiled PB treated with

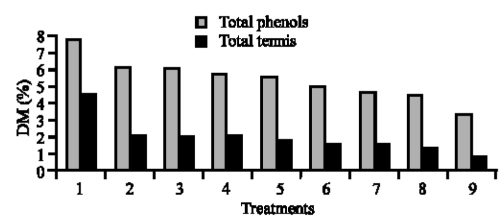

Fig. 1: Total phenols and tannins contents of dried and ensiled PB without or with different additives. 1) Dried PB under sun-light, 2) Ensiled PB without additive, 3) Ensiled PB with 3% molasses, 4) Ensiled PB with 1.5% molasses, 5) Ensiled PB with 0.15% urea and 1.5% molasses, 6) Ensiled PB with 0.15% urea and 3% molasses, 7) Ensiled PB with 4.5% molasses, 8) Ensiled PB with 0.15% urea and 4.5% molasses and 9) Ensiled PB with 0.15% urea

Table 2: Degradability coefficients of dried and ensiled PB

	a[2]±SE		b±SE		c±SE	
Treatments[1]	DM	CP	DM	CP	DM	CP
1	0.439±0.007	0.554±0.012	0.421±0.017	0.418±0.068	0.025±0.003	0.016±0.005
2	0.379±0.006	0.451±0.010	0.458±0.020	0.435±0.038	0.022±0.002	0.020±0.004
3	0.465±0.007	0.671±0.009	0.427±0.025	0.339±0.043	0.020±0.003	0.017±0.005

[1]The treatments were; 1) Dried PB under sun-light, 2) Ensiled PB without additive and 3) Ensiled PB with 0.15% urea and 1.5% Molasses. 2a = The rapidly soluble fraction, b = The potentially degradable DM or CP and c = The constant rate of degradation of b parameter (Percent h^{-1})

Table 3: DM and CP disappearances (g kg^{-1}) of PB in the rumen, small intestine and whole digestive tract

Site of disappearance	Treatment[1] 1	2	3	SEM
Rumen				
DM	531.25[a]	462.75[b]	540.00[a]	4.36
CP	587.25[a]	507.00[b]	683.25[c]	2.23
Intestine				
DM	185.00	156.75	146.75	19.78
CP	184.75[a]	141.00[ab]	135.75[b]	19.76
Whole digestive tract				
DM	618.00[a]	547.00[b]	607.75[a]	9.20
CP	663.25[a]	574.00[b]	726.75[c]	7.69

[1]The treatments were; 1) Dried PB under sun-light, 2) Ensiled PB without additive and 3) Ensiled PB with 0.15% urea and 1.5% molasses. Means in row with different superscript letters are different *p = 0.05

urea and molasses (Table 3). Lower level of intestinal CP disappearance for PB with urea and molasses was probably due to higher ruminal CP disappearance.

CONCLUSION

PB utilization as well as the feed sources is critical for a country like Iran with large feed shortage. The fresh PB must be converted into the stable forms. Natural drying under sun-light and ensiling are the most applicable processes in the area. Tannin deactivation is another important issue for better utilization of this feed source. As it was noted in this study, large differences in case of chemical composition exist between the reported results. More studies with ruminants at farm levels are highly recommended.

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