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Deactivation of tannins in raisin stalk by polyethylene glycol-6000 effect on feed intake, digestibility, and nitrogen retention in sheep

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Abstract: An experiment was conducted to assess the effects of polyethylene glycol (PEG-6000) and urea on feed intake, nitrogen retention, and nutrient digestibility in sheep fed raisin stalk. Sixteen male fat-tailed Sanjabi sheep (26 ± 0.66 kg) were randomly assigned to one of 4 dietary treatments: raisin stalk (control; RS), raisin stalk supplemented with 3% urea and 4% molasses (RSU3%); 5% urea and 7% molasses (RSU5%); or 5% urea, 7% molasses plus 5% PEG (RSU-PEG) per DM of raisin stalk. The urea and molasses were dissolved in distilled water and then mixed into a solid ingredient. Polyethylene glycol was also sprayed onto the diet with a solution of PEG in distilled water (5 g/10 mL). The results indicated that feed intake, DM digestibility, average daily gain, and nitrogen retention significantly increased due to incorporation of molasses, urea, and PEG-6000, which may be associated with the inactivation of TEPH and TET. The digestibility of CP increased from –20.5% to 78.6%, and nitrogen retention in the PEG group was significantly higher than that in the other groups ($P < 0.05$). No significant effect of urea alone was observed in the average daily gain. The negative effects of tannin on DM intake and N retention were alleviated when animals received molasses with 5% urea + 5% PEG.

Key words: Raisin byproduct, digestive characteristics, tannin, N-balance, fat-tailed sheep

1. Introduction

Ruminants can utilize inexpensive byproduct feedstuffs because of their rumen physiological adaptation (1). Agro-industrial byproducts play a potentially important role in providing animal feed that can improve livestock feeding in developing countries (2). The geographical conditions of Iran (arid and semiarid) have led to high prices of feed, particularly the cereal grains in ruminant diets. Liquid molasses is a beet byproduct that is a palatable and cheap source of energy for ruminants. In Iran, production of this byproduct is about 480,000 t/year (3), and it is readily available in many parts of the country. Generally, molasses is deficient in nitrogen (N). Thus, adding NPN sources (like urea) to molasses often improves its N status and optimizes rumen fermentation (4). Raisin stalk is a byproduct of the raisin industry. There is little scientific information available on the nutritive value of raisin stalk in animal nutrition. It has been reported that raisin stalk has negative effects on digestion and the daily growth rate of sheep (2,5), suggesting that raisin stalk could not be fed alone to sheep. The nutritive value of raisin stalk is also limited by the presence of antinutritional factors such as tannins. Although moderate levels of tannins can have beneficial responses in ruminants (6,7), high levels of tannins in feed stuffs often limit the digestibility of the diet because they are unable to form a stable protein–tannin complex at rumen pH (8,9). Tannins can also inhibit enzymatic (10,11) and microbial activity (7,12) in the rumen and suppress feed intake (6,13). Tannins that bind with dietary protein can be counteracted with a competitive agent such as polyethylene glycol (14,15). PEG may alleviate the deleterious effects of dietary tannins in tanniniferous feeds by preventing the formation of tannins and protein complexes and even releasing protein from preformed complexes (16,17).

In our previous study (in vitro) the effect of PEG + urea on digestibility and gas production was evaluated, and PEG as a tannin-complexing agent neutralized the negative effects of tannin (2). The main aim of this study was, therefore, to examine the possibilities of alleviating the negative effects of dietary tannins by supplementation with urea, simultaneous with the provision of PEG, to improve the digestibility and utilization of raisin stalk. Measurements of feed intake, digestive characteristics, nitrogen balance, and concentration of tannins in feed and feces were made to assess the effects of PEG and urea on the nutritive value of raisin stalk.

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2. Materials and methods

2.1. Animals and experimental design

The study was conducted at the Razi University sheep farm, Kermanshah province, Iran (34°18′N, 47°3′E). A total of sixteen healthy, male fat-tailed Sanjabi sheep with an average live weight of 26 kg (±0.66) were used. They were divided into 4 groups in a completely randomized design. The experiment consisted of a 60-day intake and live weight gain trial followed by a digestibility and N-balance trial.

The sheep were randomly allocated to individual pens and had free access to feed and water. Before the experimental period they were fed with oat hay ad libitum. In order to avoid toxicity problems, they were allowed a period of 4 weeks to adapt to the experimental conditions and were introduced to raisin stalk gradually before commencing the experimental period. The proportion and composition of diets are given in Table 1.

The sheep were weighed and randomly assigned to one of four dietary treatments: raisin stalk (control; RS), raisin stalk supplemented with 3% urea and 4% molasses (RSU3%), 5% urea and 7% molasses (RSU5%), or 5% urea and 7% molasses plus 5% PEG (RSU-PEG) per DM of raisin stalk.

2.2. Diets and feeding

Raisin stalks used in this experiment were obtained from a raisin producing factory in Malayer, located in west Iran. Raisin stalks are the byproduct of the raisin industry after the raisins have been removed. The chemical compositions of the diets are given in Table 2.

Raisin stalk had a relative low concentration of CP. The total extractable phenol and TET of raisin stalk (g acid tannic/kg DM raisin stalk) was 139 and 21.3 g/kg, respectively.

The diet was prepared by mixing raisin stalks with distilled water at a ratio of 20% volume/weight. The urea

Table 1. Proportion and composition of diets (g/kg DM of diet).

Ingredients	Treatments			
	RS*	RSU (3%)	RSU (5%)	RSU-PEG
Raisin stalk	1000	930	880	830
Urea	-	30	50	50
Molasses	-	40	70	70
PEG	-	-	-	50

*RS: raisin stalk (control); RSU3%: raisin stalk supplemented with 3% urea and 4% molasses; RSU5%: raisin stalk supplemented with 5% urea and 7% molasses; RSU-PEG: raisin stalk supplemented with 5% urea, 7% molasses plus 5% PEG per DM.

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

Attributes	Treatment groups			
	RS*	RSU3%	RSU5%	RSU-PEG
Dry matter (DM)	72.66	73.29	72.68	72.53
Organic matter (OM)	93.05	93.15	93.22	89.24
Crude protein (CP)	8.6	16.18	20.55	19.65
Ether extract (EE)	2.2	2.05	1.94	1.87
Crude fiber (CF)	13.41	12.52	11.85	11.38
Ash	6.95	6.85	7.78	10.76
Total extractable phenol (TEPH)	13.9	12.93	12.23	11.54
Total extractable tannin (TET)	2.13	1.98	1.87	1.77

*RS: raisin stalk (control); RSU3%: raisin stalk supplemented with 3% urea and 4% molasses; RSU5%: raisin stalk supplemented with 5% urea and 7% molasses; RSU-PEG: raisin stalk supplemented with 5% urea, 7% molasses plus 5% PEG per DM.

and molasses were dissolved in distilled water and then mixed with the other ingredients of the diet. Polyethylene glycol was also sprayed on the diet with a solution of PEG in distilled water (5 g/10 mL). The diet was offered in two equal meals at 0900 and 1400. The sheep had free access to mineralized salt blocks and fresh water throughout the experiment.

2.3. Measurements and chemical analyses

The digestibility trial was conducted after 4 weeks of adaptation of the animals to experimental conditions. The sheep were housed in metabolism crates, and after an adaptation period of 4 days, the feed refusals, feces, and urine excreted by each animal were recorded daily at 0830 over the 7 days. The daily feces voided were weighed and sampled, collected for a 7-day period, and then stored at −20 °C. The urine was collected in a bucket containing 50 mL of 0.1 M sulfuric acid so that the final pH of the urine was below 3.0 (8). Daily urine production was measured volumetrically, and a 10% portion was taken and stored at −20 °C. Samples of feed offered and refusals were collected daily from each animal and stored at −5 °C. At the end of the measurement period, daily urine samples from each animal were analyzed for CP content. Feed, refusals, and daily feces samples were dried and ground to pass through a 1 mm screen. Dry matter (DM) content of food, refusals, and feces was determined by drying the sample in an oven at 100 °C overnight. The crude ash was determined at 550 °C for 7 h, and ether extract (EE), crude fiber (CF), and crude protein (CP) were determined by AOAC (18). For the digestibility trial, the quantities of DM and individual nutrients consumed and chemical composition of the feces for each nutrient were evaluated. Digestibility coefficients of DM and nutrients were calculated according to the following equation:

$$\frac{\text{Nutrient consumed} - \text{nutrient in feces}}{\text{Nutrient consumed}}$$

The sheep were weighed at the end of the digestibility trial. The average daily gain was calculated for each animal.

Total extractable phenol (TEPH) was determined according to a modified version of the method described by Julkumen-Tiitto (19) using the phenol Ciocalteu (Folin reagent). Samples of raisin stalk, feces, and food refusals were ground to particular size of 1 mm before chemical analysis. A representative 0.1-g sample was extracted with 5 mL of acetone (70%) for 35 min at room temperature. After 35 min, the absorbance of blanks and tannin acid standard solutions at 725 nm were read using a spectrophotometer. The concentration of TEPH was calculated using regression (2). Total extractable tannins (TET) were estimated indirectly after being absorbed into insoluble polyvinyl pyrrolidone (PVP) by a modified method of Makkar et al. (20). The mixture was then centrifuged at 3000 rpm for 5 min, and the supernatant was used to determine the total remaining phenols. Concentration of TET was calculated by subtracting the TEPH remaining after PVP treatment from TEPH.

2.4. Statistical analysis

This experiment was carried out as a completely randomized design (CRD) with the same replicates for each treatment. Data were analyzed by general linear models (GLM) using procedures of SAS

$$Xij = \pi + \tau_j + \varepsilon_{ij},$$

where *Xij* is the jth observation of the ith treatment, π is the population mean, τj is the treatment effect of the ith treatment, and ε*ij* is the random error.

The CRD with four replicates and treatment means was compared by Tukey's test.

The results obtained from average daily gain, digestibility, and nutrient intake of DM, CP, OM, CF, EE, and N retention were compared by one-way analyses of variance using Minitab statistical software (version 15).

3. Results

The average feed intake and daily gains are given in Table 3. Dry matter intake was affected by treatments, and PEG increased the amount of CP consumed by sheep ($P < 0.05$). Intake of OM in RSU-PEG group was significantly higher than in the other groups. There was a linear increase in CP intake due to increasing CP concentration in the diet. The highest increase in CP intake was obtained with RSU-PEG, resulting in increased average daily gain.

The treatments had a positive effect on nutrient digestibility. The sheep in RSU3% had significantly greater values of apparent digestibility of CP and EE than the sheep in RS (control). The other values (DM, OM, and CF digestibility) also increased in RSU3%. The DM, CP, and OM digestibility of the diet were higher in the 5% urea group than in the RS group, but there were no differences between RSU groups. The digestibility of CP, CF, and EE was significantly higher in RSU-PEG than in the other groups. Dry matter and OM digestibility in RSU-PEG were similar to those in RSU3% and RSU5%. The CP digestibility of the diet in RSU-PEG increased approximately 14.7% and 42.1% compared with RSU3% and RSU5%, respectively. Nitrogen intake (g/day) was significantly lower for the sheep in RS than for those in other groups (Table 5). The sheep on raisin stalk containing urea (RSU) excreted more fecal N than those on the PEG-supplemented diet (% of N intake). Excretion of fecal N decreased by 21.32% in sheep on RSU-PEG. Urinary N excretion (g/day) in the RSU-PEG group tended to increase; however, as a proportion of N intake it was similar to other urea-supplemented diets. The sheep in both the RSU3% and RSU5% groups

Table 3. Effect of PEG-6000 and urea on nutrient intake and live weight changes in fat-tailed sheep.

Attributes	Treatment groups				SEM
	RS*	RSU3%	RSU5%	RSU-PEG	
Nutrient intake					
DM: (g/day)	216.9[a]	457.2[b]	534[b]	1246.6[c]	26.85
(g/kg $W^{0.75}$)	20.71[a]	41.73[b]	46.38[b]	102.41[c]	2.81
CP (g/day)	17.18[a]	77.32[b]	115.37[c]	247.09[d]	3.98
OM (g/day)	201.8[a]	425.1[b]	498.3[b]	1110.6[c]	24.91
EE (g/day)	5.55[a]	10.62[b]	11.45[b]	23.74[c]	0.29
CF (g/day)	34.02[a]	65.39[b]	64.18[b]	145.90[c]	2.25
Initial live weight (kg)	26	25.83	26.67	25.5	0.47
Final live weight (kg)	21.83[a]	24.5[b]	25.67[b]	28.67[c]	0.69
Average daily gain (g/day)	−148.81[a]	−47.62[b]	−35.71[b]	113.10[c]	15.2

Values with different superscripts are significantly different (P < 0.05) within rows.
*RS: raisin stalk (control); RSU3%: raisin stalk supplemented with 3% urea and 4% molasses; RSU5%: raisin stalk supplemented with 5% urea and 7% molasses; RSU-PEG: raisin stalk supplemented with 5% urea, 7% molasses plus 5% PEG per DM.

Table 4. Effect of PEG-6000 and urea on nutrient digestibility in fat-tailed sheep (%).

Apparent digestibility	Treatment groups				SEM
	RS*	RSU3%	RSU5%	RSU-PEG	
Dry matter	50.24[a]	57.64[ab]	59.53[bc]	65.85[c]	2.44
Crude protein	−20.5[a]	55.29[b]	63.42[b]	78.61[c]	3.8
Organic matter	51.36[a]	57.82[ab]	60.71[b]	65.31[b]	2.46
Crude fiber	60.11[a]	67.07[ab]	58.37[a]	74.99[b]	2.7
Ether extracted	51.76[a]	60.31[bc]	56.57[ab]	64.75[c]	1.89

Values with different superscripts are significantly different (P < 0.05) within rows. *RS: raisin stalk (control); RSU3%: raisin stalk supplemented with 3% urea and 4% molasses; RSU5%: raisin stalk supplemented with 5% urea and 7% molasses; RSU-PEG: raisin stalk supplemented with 5% urea, 7% molasses plus 5% PEG per DM.

exhibited lower values for N retention when compared with the RSU-PEG group. The minimum and maximum values of N retention were observed in sheep in the RSU and RSU-PEG groups, respectively.

A reduction in TEPH and TET content of feed refusal was observed in the RSU-PEG group (Table 6).

The TEPH and TET concentration ranged between 4.2 and 15.96 and 0 and 6.66 mg/g DM, respectively. The lowest concentrations of TEPH and TET in feed refusal were obtained in the RSU-PEG group. The TEPH and TET concentrations in feed refusal in the RS and RSU groups were similar. There were no differences in fecal TEPH among the treatment groups. The concentration of TET in feces voided by sheep in the RS, RSU3%, and RSU5% groups was nil; however, it was 1.9 (%DM) in the RSU-PEG group.

Table 5. Effect of dietary supplementation with PEG-6000 and urea on nitrogen balance in fat-tailed sheep.

Nitrogen balance	Treatment groups				SEM
	RS*	RSU3%	RSU5%	RSU-PEG	
Intake (g/day)	2.75[a]	12.37[b]	18.46[c]	39.53[d]	1.2
Voided in feces:					
(g/day)	3.2[a]	5.48[b]	6.73[b]	8.43[c]	0.7
(% of N intake)	116.36[a]	44.3[b]	36.45[b]	21.32[c]	3.17
Voided in urine:					
(g/day)	2.55[a]	3.72[a]	5.08[a]	11.43[b]	0.91
(% of N intake)	92.72[a]	30[b]	27.51[b]	28.91[b]	2.87
Retention:					
(g/day)	−3[a]	3.17[b]	6.65[b]	19.67[c]	1.2
(% of N intake)	−109.08[a]	25.7[b]	36.04[b]	49.77[c]	8.89

Values with different superscripts are significantly different ($P < 0.05$) within rows.
*RS: raisin stalk (control); RSU3%: raisin stalk supplemented with 3% urea and 4% molasses; RSU5%: raisin stalk supplemented with 5% urea and 7% molasses; RSU-PEG: raisin stalk supplemented with 5% urea, 7% molasses plus 5% PEG per DM.

Table 6. Effect of PEG-6000 and urea on TEPH and TET concentrations of feed refusal and feces (%DM).

Attributes	Treatment groups				SEM
	RS*	RSU3%	RSU5%	RSU-PEG	
TEPH:					
Feed refusal	15.96[a]	15.28[a]	15.9[a]	4.2[b]	0.62
Feces	0.73	0.5	1	1.36	0.12
TET:					
Feed refusal	6.66[a]	5.1[a]	3.9[a]	0[b]	0.85
Feces	0	0	0	1.9	

Values with different superscripts are significantly different ($P < 0.05$) within rows.
*RS: raisin stalk (control); RSU3%: raisin stalk supplemented with 3% urea and 4% molasses; RSU5%: raisin stalk supplemented with 5% urea and 7% molasses; RSU-PEG: raisin stalk supplemented with 5% urea, 7% molasses plus 5% PEG per DM.

4. Discussion

The chemical composition of raisin stalk obtained in this study was similar to the previously reported composition (5). The lowest intake of raisin stalk by sheep was observed in the control diet. The low intake of tannin-rich feeds is generally attributed to their astringent taste. In addition to the unpleased taste, the lower rate of digestion in response to tannins (1,5) could be responsible for the lower intake (18). Low concentrations of condensed tannins (20–45 g CT/kg DM) reduce rumen forage protein degradation due to reversible binding to these proteins, which reduces the populations of proteolytic rumen bacteria (7) and nutrient digestibility (21). The effect of hydrolyzable tannins is also variable and mainly dependent on the quantity consumed (13,22). In the present study supplementation of raisin stalk with urea increased the daily intake, although increasing the amount of urea (up to 5%) did not result in a further increase in DMI. Similarly, Frutos et al. (22) found no reduction in voluntary feed intake among sheep provided with feed containing soya bean meal treated with hydrolyzable tannins. Maximum consumption of raisin stalk by sheep was observed in the group supplemented with PEG. This polymer is known to alleviate the deleterious effects of dietary tannins on feed intake (23) and affect nutrient digestibility (19) in ruminants fed tanniniferous feeds. Ben Salem et al. (10) reported that PEG increased DM intake of *Acacia cyanophylla* by Barbarine lambs, suggesting that PEG offered with or without urea exerts a direct positive effect on microbial growth and activity. Additionally, PEG-supplemented raisin stalk might allow for a balanced supply of energy from molasses and nonprotein nitrogen from urea, resulting in an increase in raisin stalk consumption by sheep. This is in agreement with previous findings in sheep given PEG-treated foliage of *Acacia cyanophylla* (14). The increased DM digestibility was in agreement with Hatch and Beeson (24), who found DM digestibility increased when molasses replaced either 100 or 150 g/kg of the corn in diets containing NPN (urea) for steers. Broderick and Radloff (25) found that digestibility coefficients of DM, OM, NDF, and ADF were enhanced linearly when different levels of molasses (0, 40, 80, or 120 g/kg of DM diet) were added at the expense of corn in rations for dairy cows.

In the present study, minimum values for DM, OM, CP, CF, and EE digestibility were observed in sheep fed the RS (control) diet. Considering the low dietary CP concentration and the presence of tannins in raisin stalk, it seems that sufficient N was not available to support ruminal fermentation in the control group. Several studies have shown the ability of tannins to reduce the digestibility of tanniniferous diets (16,26). Tannins exert this effect on proteins, hemicelluloses, cellulose, starch, and pectin (22) and reduce populations of proteolytic rumen bacteria (27). Although provision of urea increased CP and CF digestibility of raisin stalk, drastic increases in these values were observed with supplementation with PEG. The improvements in digestibility could be due to both the increased rate and extent of digestion in the rumen as a result of PEG binding with condensed tannins and preventing the formation of indigestible tannin-protein complexes (24,28). Similar results in which PEG supplementation neutralized the negative effects of tannins along the digestive tract in goats have been reported (27).

The beneficial effects of PEG on raisin stalk digestibility were reflected in nitrogen utilization. Following this supplementation, increasing N intake (39/53 g/day) was associated with decreasing N loss, mainly via feces and urinary excretion (% N intake). The lower fecal N losses observed in sheep fed PEG-treated raisin stalk may indicate that some tannin–protein or tannin–ammonia complexes are dissociated in the gastrointestinal tract, resulting in increased absorption of N. This results in a drastic increase in N retention compared to the other treatments. This may be attributed to deactivation of tannin by PEG, causing enhancement of protein fermentation in the rumen, increased ammonia concentration, and improved efficiency in N utilization (2). Similarly, by comparing values for tannin-rich diets Makkar (17) reported positive effects on N utilization in ruminant animals fed a PEG-supplemented diet. The additional N provided by urea and released from the disassociation of protein–tannin complexes by PEG could be metabolized by the animal (10). Such a trend suggests that tannins were the main causative factor for poor utilization of N in raisin stalk by the sheep.

Dietary tannins can also reduce the palatability of the feed (27). The negative effects of tannin on appetite could be caused by a reaction between tannins and the salivary mucoproteins, or through a direct reaction with the taste receptors, provoking an astringent sensation (22). Thus, tannins may affect the selection of feed by ruminants. This effect was observed in the present study through a reduction in TEPH and TET concentrations in feed refusal in the PEG-supplemented group compared to the unsupplemented group (4.2% and 0.0% vs. 15.96% and 6.66% DM, respectively).

The concentrations of TEPH and TET excreted in the feces were negligible in the control and urea-supplemented groups. This is in agreement with previous findings in sheep and goats fed tannin-rich *Acacia soligna* leaves (29). They showed a virtual absence of tannins in the feces of the animals. The extractable tannins bind proteins and are covered in fiber fractions, explaining the negligible presence of extractable tannins in the fecal samples (19). Furthermore, tannins or their breakdown products can be absorbed from the gut and inhibit metabolism directly, and this could be the main effect of tannins (8). PEG, as a tannin-binding agent, prevents protein complexes and even releases protein from preformed tannin–protein complexes (15,30). In the present study, supplementation with PEG resulted in TET excretion in the feces of the sheep, which was associated with increasing CP and CF digestibility and N retention, as discussed earlier.

In conclusion, the results of this study suggest that raisin stalk has low digestibility and nutritive value in sheep, probably due to the tannins. The presence of tannins in raisin stalk is associated with a reduction in nutrient digestibility, mainly CP and N retention. This deleterious effect of tannins can be neutralized by urea and PEG-6000 supplementation. The improvement in nutrient digestibility and N retention with PEG emphasizes the negative effects of tannins on digestibility. More research is needed to find the optimal levels of urea, molasses, and PEG.

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