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# Effect of Processing Protein Supplements with Tannin Extracted from Pistachio by-Products on Performance of Holstein Dairy Cows in Early Lactation

Research Article

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## ABSTRACT

This experiment was conducted to evaluate the effects of processing protein meals by tannin extracted from pistachio by-product on apparent total tract digestibility, ruminal fermentation and performance of dairy cows. For tannin extraction, one kilogram of sun-dried pistachio by-products was immersed in four liters of water for 48 h. Then, pistachio by-products were removed from the water and the extract obtained was sprayed on protein supplements (canola meal and soybean meal) with an equal ratio of 1:1 (L/kg) and was dried in the shade. Eight nulliparous Holstein dairy cows with 27 ± 49 days in milk (DIM) and milk production of 38 ± 4 kg/d were used in the form of a replicated Latin square (4×4) design. The experiment was performed in four 21-day periods consisting of 14 days for adaptation and 7 days for recording. The experimental treatments were as follows: 1) ration based on soybean meal, 2) ration based on soybean meal processed with the extracted tannin by water-extracted tannin from pistachio by-product, 3) ration based on canola meal and 4) ration based on canola meal processed with water-extracted tannin from pistachio by-product. Spraying extracted tannin on protein supplement increase concentration by 370 and 246% in soybean meal and canola meal, respectively. Intake of dry matter (DM) and total tract apparent digestibility were not affected by treatments. Although daily milk production (kg/d) and milk protein percentage were not affected by treatments, cows fed tannins produced more milk and milk protein. Tannins decreased rumen ammonia concentration only in the canola diets. Soybean treatment had the highest and processed canola treatment with tannin had the lowest rates of ammonia nitrogen in the rumen. Treated protein supplements with tannins decreased ruminal pH, soybean treatments showed the highest and processed canola treatment had the lowest pH rates with tannin. Although blood urea nitrogen was not affected by the treatments, processing the rations with tannin reduced blood urea nitrogen linearly however; other blood metabolites were not affected by treatments. Results of this study show that under our experimental conditions, processing protein supplements with tannin had no negative effect on animals, Further experiments should be done in this regard and on the rations containing higher levels of tannin.

KEY WORDS dairy cows, extract tannin, pistachio by-product, protein supplement.

## INTRODUCTION

Iran is among countries which are mainly located in arid and semiarid regions in which annual rainfall is low. Shortage of livestock feed is one of the basic and remarkable problems in the livestock industry and to compensate for this deficiency, utilizing new sources of food and wastes of agricultural products and also their proper processing for livestock feed are one of the strategies in developing countries. Annually, more than 400 thousand tons of byproducts

derived from pistachio peeling are produced in Iran (Bagheripour *et al*. 2008). On the other hand, Since FAO has recommended a global ban on the feeding of mammalian meat and bone meal to cattle, sheep and goats (FAO, 2001) protein supplements like soybean meal (SBM) or canola meal (CM) has been most commonly used for ruminants. The major anti-nutritional factor of pistachio by-product in livestock is its high amount of tannin. (Mahoney and Rodriguez, 1996). Bate-Smith and Swain (1962) defined tannins as naturally occurring water-soluble polyphenolic compounds with a molecular weight between 500 and 3000 capable of precipitating alkaloids as well as gelatin and other proteins from aqueous solutions. Tannins divided into two groups: proanthocyanidin or condensed tannins and acid gallic polyesters anddyfinic hydro hegzaacid or hydrolysable tannins (galo tannin and elagy tannins, respectively) (Makkar 2003). Available tannins in forages have both negative and positive roles in the nutritional value of forages and depend on tannin level and type and the nature of the ration (Frutos *et al*. 2004). Low and moderate levels of tannin in the ration increase nitrogen retention in the sheep and cows. Moderate levels of tannin (less than 4%) in legumes have a positive effect on ruminants and optimize growth and increase milk production (Hove, 2001); however, the inverse relationship between high tannin level in the forage and palatability, feed intake and digestibility in ruminants has been well established (Ben Salem *et al*. 2000; Silanikove *et al*. 1996). So, the aim of this experiment was to investigate the effect of processing protein supplements with the extracts derived from tannins in pistachio by product on the digestibility of nutrients and performance in lactating Holstein cows in early lactation.

## MATERIALS AND METHODS

For tannin extraction, one kilogram of sun-dried pistachio by-products (the pistachio by-product were collected from pistachio peeling terminal in Kashmar City in 2009) was immersed in four liters of water for 48 h. Then, pistachio by-products were removed from the water and the obtained extract was sprayed on protein supplements (canola meal and soybean meal) with equal ratio of 1:1 (L/kg) and was dried in the shade. After drying, the protein supplements were utilized for making concentrate of experimental treatments. To measure the amount of total tannin and total amount of phenolic compounds, the folin-ciocalteau method was used (Makkar, 2000) and to measure condensed tannin, the Porter method (1988) was applied. To investigate the effects of the extracted tannin from pistachio by-products on the performance of lactating Holstein cows, 8 primiparous Holstein dairy cows with the 27 ± 49 DIM and milk production of 38 ± 4 kg/d were used in the form of a replicated latin square (4×4) design. The experiment was performed in four 21-day periods consisting of 14 days for adaptation and 7 days for recording. Animals were kept in individual tie stalls in a barn, protected from rain and wind and equipped with individual troughs to facilitate quantitative measurement of feed intake. The experimental treatments were as follows: 1) ration based on soybean meal, 2) ration based on soybean meal processed with the extracted tannin from pistachio by-product, 3) ration based on canola meal and 4) ration based on canola meal processed with extracted tannin from pistachio by-product. The experimental rations were formulated with the ratio of 37% forage (20% alfalfa hay and 17% corn silage) and 63% concentrate based on the requirements food NRC (2001). Ingredient and chemical composition of experimental rations are shown in Table 1.

**Table 1** Feed ingredients of the experimental diets (%)

| Item | Treatment | | | |
|---|---|---|---|---|
| | T1 | T2 | T3 | T4 |
| Ingredients, % | | | | |
| Alfalfa hay | 20 | 20 | 20 | 20 |
| Corn silage | 17 | 17 | 17 | 17 |
| Whole cotton seed with lint | 7.0 | 7.0 | 7.0 | 7.0 |
| Corn | 21 | 21 | 21 | 21 |
| Soybean meal | 19 | 0 | 0 | 0 |
| Soybean + extract tannin | 0 | 19 | 0 | 0 |
| Canola meal | 0 | 0 | 24 | 0 |
| Canola meal + extract tannin | 0 | 0 | 0 | 24 |
| Barley grain | 7 | 7 | 7 | 7 |
| Wheat bran | 6 | 6 | 1.5 | 1.5 |
| Fat | 1.5 | 1.5 | 1 | 1 |
| Limestone | 0.5 | 0.5 | 0.5 | 0.5 |
| Vitamin-mineral Mix[a] | 0.8 | 0.8 | 0.8 | 0.8 |
| Salt | 0.2 | 0.2 | 0.2 | 0.2 |
| Chemical composition, % of dry matter (DM) | | | | |
| Organic matter (OM) | 93.6 | 93.6 | 93.4 | 93.4 |
| Crude protein (CP) | 17.9 | 17.9 | 17.6 | 17.6 |
| Acid detergent fiber (ADF) | 19.7 | 19.7 | 22 | 22 |
| Neutral detergent fiber (NDF) | 30.5 | 30.5 | 32 | 32 |
| Nonfiber carbohydrates (NFC)[b] | 42 | 42 | 41 | 41 |
| Ether extract | 5 | 5 | 5.3 | 5.3 |
| Net energy for lactation ($NE_L$) | 1.6 | 1.6 | 1.59 | 1.59 |
| Ca | 1 | 1 | 0.5 | 0.5 |
| P | 1 | 1 | 0.6 | 0.6 |
| Tannins | 1.39 | 1.98 | 1.74 | 2.45 |

T1) ration based on soybean meal; T2) ration based on soybean meal processed with the extracted tannin by water from pistachio by-product; T3) ration based on canola meal and T4) ration based on canola meal processed with the extracted tannin by water from pistachio by-product.
[a] The mix contained (/kg of premix; DM basis): vitamin A: 330000 IU; vitamin D: 60000 IU; vitamin E: 1000 IU; Ca: 160 g; P: 85 g; Na: 63 g; Mg: 45 g; Zn: 2100 mg; Mn: 1500 mg; Cu: 535 mg; Se: 12 mg and I: 45 mg.
[b] NFC calculated as: 100 – (CP+Ash+NDF+EE).

Cows were fed twice daily (06:00 and 18:00 h) sampling from feed, orts and feces was performed on the last 7 days

of each period. Feed intakes and feed refusals were collected before the morning feeding and weighed daily during the measurement period. Dry matter intake was calculated by difference between the total amount of DM offered and refused. Fecal samples of each cow were collected through the 5-day collection periods and then dried in an oven. Daily dried samples were ground and later composited for 5-day periods.

Feeds and orts were sampled daily during the collection period and were composited by period. Composite samples of the total mixed ration (TMR), feed refusal and feces were dried in an oven, then ground to pass through a 2 mm screen and stored for later analysis. Rumen fluid samples were taken from animals by stomach tube with a vacuum pump 2 h after the morning feeding on days 18 and 19 .The pH was measured immediately with a portable digital pH meter (METROHM 691).

Approximately 100 mL of ruminal content was strained through four layers of cheesecloth. A subsample of 5 mL was combined with 1 mL of HCl 0.2 N for $NH_3$-N analysis. Ruminal subsamples were frozen at -20 ˚C until laboratory analyses. Blood samples were taken from the jugular vein (10-15 mL) on day 20, just 2 h after the morning feeding, centrifuged and the serum was recovered and stored at -20 ˚C. Cows were milked three times daily at 05:00, 13:00 and 21:00 h.

Milk production was recorded daily for each animal. A daily composite milk sample from the morning, noon and afternoon milking was taken during the collection period and fresh subsamples were analyzed daily for chemical composition. Animals were weighed on day 21 before feeding, and then the diet given to each cow was changed. Food, orts and feces was determined by drying in an oven at 100 ˚C to a constant weight (AOAC, 1990). Ash (method 942.05) and CP (Kjeldahl N×6.25) was determined by the block digestion method using copper catalyst and steam distillation into boric acid (method 2001.11) on 2100 Kjeltec distillation unit as described in AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by Van Soest *et al*. (1991). The sodium sulphite and α-amylase were not used and both NDF and ADF were expressed exclusive of residual ash. CP fractions were determined procedure.

For tannin assay samples, were dried in an oven at 40 ˚C to constant weight to minimize changes in tannin content and activity (Makkar, 2000). Dried samples were ground to pass a 2 mm sieve and then 0.5 mm sieve. Samples (200 mg) were extracted in four replicates in 70%, (v/v) aqueous acetone (Makkar, 2000) overnight at 4 ˚C. After centrifugation (3000 g/min, 4 ˚C, 10 min), condensed tannins (CT) were measured on supernatant using the butanol-HCl reagent (Porter *et al*. 1986).

Total phenolic compounds (TP) and total tannins (TT) were determined by folin-ciocalteu reagent using tannic acid as a standard (Makkar *et al*. 2000).

The values of TP and TT were expressed as tannic acid equivalent and CT as leucocyanidin equivalent.

Digestibility of organic matter, crude protein, neutral detergent fiber and acid detergent fiber was determined by measuring their concentrations and the concentrations of acid insoluble ash (AIA) as an internal marker in the feed and fecal samples.

The $NH_3$-N concentration of rumen fluid samples was analyzed. Blood samples were centrifuged at 3000 × g for 10 min, then serum was separated and frozen at -20 ˚C. Serum urea N, glucose and protein were determined using an autoanalyzer (Biosystems A 15; 08030 Barcelona, Spain).

Milk samples were analyzed for protein, fat, lactose, solid not fat and total solids with a Milko-Scan 605 analyser (Foss Electric, Hillerød, Denmark).

**Statistical analysis**

The mixed procedure of SAS (2003) was used to analyze data for a latin square design. The model included treatment effects, period effect, random effects of cows and experimental error. Least squares means procedure (LSMEANS) was used to detect the difference between dietary treatments. Data were analyzed using the following statistical model:

$$Y_{ijk} = \mu + T_i + P_j + C_K + \varepsilon_{ijK}$$

Where:

$Y_{ijk}$: dependent variable.

$\mu$: overall mean.

$T_i$: effect of treatment (i=1, 2, 3 or 4).

$P_j$: effect of period (j=1, 2, 3 or 4).

$C_K$: random effect of cow.

$\varepsilon_{ijK}$: random residual error.

## RESULTS AND DISCUSSION

The tannins concentrations in pistachio by-product and in non-supplemented and tannin-supplemented treatments were 5.73, 1.19, 4.30, 2.34 and 6.74, respectively. Spraying extract tannin on protein supplement increase concentrations by 370% and 246% in soybean meal and canola meal, respectively (Table 2). Processing protein supplements with tannins did not affect the DMI (Table 3) or nutrient digestibility (Table 4).

Although daily milk production was not affected by experimental treatments, the cows fed the processed ration with tannin numerically produced more milk (P>0.05).

**Table 2** The amount of compounds phenols in pistachio by-product and protein supplements

| Item (% DM) | TCP | TT | CT |
|---|---|---|---|
| Pistachio by-product | 9.93 | 5.73 | 1.47 |
| Soybean meal | 1.68 | 1.19 | 0.73 |
| Soybean meal with extract tannin | 5.61 | 4.3 | 0.82 |
| Canola meal | 3.01 | 2.34 | 0.68 |
| Canola meal with extract tannin | 7.29 | 6.74 | 0.79 |

TCP: total compounds phenols; TT: total tannins and CT: condensed tannin.

**Table 3** Effect of treatment on dry matter intake, milk yield and composition

| Item | Treatment | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | SED |
| Intake of DM (kg/d) | 23.66 | 23.11 | 23.01 | 23.4 | 0.35 |
| Milk production (kg/d) | 35.5 | 35.2 | 35.2 | 35.7 | 0.80 |
| 4 % fat corrected milk (FCM) (kg/d) | 32.67 | 32.23 | 32.48 | 32.51 | 0.93 |
| Milk composition (%) | | | | | |
| Fat | 3.57 | 3.55 | 3.5 | 3.48 | 0.197 |
| Protein | 2.79 | 2.97 | 2.93 | 3.04 | 0.095 |
| Lactose | 5.68 | 5.63 | 5.62 | 5.57 | 0.066 |
| Solid not fat | 8.96 | 8.85 | 8.89 | 8.79 | 0.058 |
| Total solids | 12.53 | 12.40 | 12.39 | 12.27 | 0.23 |
| yield (kg/d) | | | | | |
| Fat | 1.23 | 1.21 | 1.23 | 1.24 | 0.137 |
| Protein | 0.98 | 1.05 | 1.04 | 1.07 | 0.098 |
| Lactose | 2.00 | 1.99 | 2.02 | 1.97 | 0.144 |

T1) ration based on soybean meal; T2) ration based on soybean meal processed with the extracted tannin by water from pistachio by-product; T3) ration based on canola meal and T4) ration based on canola meal processed with the extracted tannin by water from pistachio by-product.
FCM: fat-corrected milk.
SED: standard error deviation.

**Table 4** Effect of treatment on apparent total tract digestibility of diets

| Item (% DM) | Treatments | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | SED |
| Organic matter (OM) | 73.03 | 70.57 | 71.86 | 75.11 | 2.45 |
| Crude protein (CP) | 72.62 | 71.64 | 72.84 | 71.30 | 2.28 |
| Neutral detergent fiber (NDF) | 57.13 | 56.81 | 53.58 | 52.67 | 2.05 |
| Acid detergent fiber (ADF) | 57.68 | 56.57 | 54.20 | 57.97 | 2.20 |

T1) ration based on soybean meal; T2) ration based on soybean meal processed with the extracted tannin by water from pistachio by-product; T3) ration based on canola meal and T4) ration based on canola meal processed with the extracted tannin by water from pistachio by-product.
SED: standard error deviation.

The mean milk production for soybean meal, soybean meal processed with the extracted tannin from pistachio, canola meal, canola meal processed with the extracted tannin from pistachio by product were 35.62, 36.12, 35.60 and 35.92 respectively (Table 3). Processing protein supplements with tannins had no significant effects on percentage of milk protein (P>0.05).

The mean of milk protein concentration for treatments were 2.79, 2.97, 2.93 and 3.07, respectively. Tannins had no effect on milk protein (Table 4).

Concentration of rumen ammonia nitrogen was affected by extracted tannins in the ration (P<0.05); soybean treatment had the highest and processed canola treatment with tannin had the lowest rates of ammonia nitrogen in the rumen (Table 5). Although no significant differences were observed in this experiment between soybean and soybean processed with tannins, the ration containing tannin reduced the amount of ammonia nitrogen in the rumen.

Rumen pH was affected by treatments (P<0.05); soybean treatments showed the highest and processed canola treatment had the lowest pH rates with tannin (Table 5). Although blood urea nitrogen was not affected by the treatments (P>0.05), processing the rations with tannin has a tendency to be different among treatments. However, other blood metabolites were not affected by treatments (Table 5).

There are various results obtained from the amount of phenolic compounds in pistachios. Labavitch *et al.* (1982) reported that the amount of total compound phenolic (TCP) in maturity periods varied between 5.3 and 7.4% DM. Bagheripour *et al.* (2008) showed that the amount of TCP, total tannin phenols (TTP) and condensed tannin (CT) from PB was 14.11, 9.71 and 0.91%, respectively. Bohluli (2007) expressed that the amounts of phenolic compounds and total tannin pistachio by-product were 8.6 and 4.1, respectively.

**Table 5** Effect of treatment on ruminal parameters and blood metabolites

| Item | Treatments | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | SED |
| Ruminal parameters | 6.71[a] | 6.47[b] | 6.74[a] | 6.46[b] | 0.075 |
| Ruminal fluid pH | | | | | |
| Ruminal $NH_3$-N (mg/dL) | 22.17[a] | 20.76[a] | 21.78[a] | 19.47[b] | 0.98 |
| Blood metabolites (mg/dL) | | | | | |
| Glucose | 51.82 | 53.80 | 53.45 | 51.90 | 3.39 |
| Protein | 8.37 | 8.41 | 8.22 | 8.28 | 0.283 |
| Serum urea N | 18.84 | 18.20 | 18.82 | 17.65 | 0.76 |
| Albumin | 3.76 | 3.65 | 3.76 | 3.95 | 0.103 |

T1) ration based on soybean meal; T2) ration based on soybean meal processed with the extracted tannin by water from pistachio by-product; T3) ration based on canola meal and T4) ration based on canola meal processed with the extracted tannin by water from pistachio by-product.
SED: standard error deviation.

Similar to the present research, Mokhtarpour (2009) reported that TCP, total tannin (TT), condensed tannin (CT) from pistachio by-product was 10.00, 5.2 and 1.15%, respectively. This inconsistency might be due to varieties, maturity age and methods of drying systems. Tannins are one of many compounds within canola meal and their presence makes it dark. There are few studies on spraying tannin extract on protein supplements. Hervas *et al*. (2000) reported that dissolving 1, 5, 10, 15 and 25 g of commercial tannic acid in 100 mL distilled water and spraying that on SBM increased the amount of tannic acid to 1, 4.8, 9.1, 13 and 20 g/100 g, respectively.

The rations containing tannin generally reduce feed consumption. Astringent taste of tannins reduces food palatability and as a result, causes animals to avoid feed consumption. Toxicity and low discharge rate of digested materials from rumen are effective in reducing feed consumption (Kumar and Singh, 1984; Provenza, 2000). Studies have demonstrated that the minimum amount of tannins in feed that affect feed consumption in ruminants is 2% of dry matter (Kumar and Singh, 1984). Shakeriand Fazaeli (2005) reported that levels of 10 and 20% of dry matter of pistachio by-products (dried) in male sheep rations had no negative effect on feed consumption; however, using 30% of dry matter of these by-products in a ration caused significant reduction in feed consumption ($P<0.05$). These researchers also attributed reduction of feed consumption to the existence of phenolic compounds and tannins in pistachio by-products. Bhatta *et al*. (2000) aimed at investigating tannin effects on the performance of dairy cattle and applied Tamarindus indica hulls containing 14.5% tannin in dry matters in the ration with the maximum of 7.5% of its dry matter. These researchers observed no changes in dry matter consumption of rations and concluded that tannin concentration in the rations (0.74% of dry matter) was not sufficient for affecting feed consumption in cows.

In this research, since tannin level of experimental treatments was not high, tannins were not expected to have a negative effect on feed consumption and feed consumption was not affected by experimental treatments.

In single-stomach mammals (Jansman, 1993) or in ruminants (Silanikove *et al*. 1996), one the negative effects of tannin on using nutrients is the reduction of pancreatic enzyme activity. The most important effects of anti-nutritional tannins is reducing digestibility of crude protein. This effect of tannins is caused by reduction of protein availability degree and reduction of enzyme activity in the gastrointestinal tract (Kumar and Singh, 1984). Several studies have shown that the presence of tannin in rations causes reduction in digestibility of protein (Ben Salem *et al*. 2000; Silanikove *et al*. 1996; Waghorn and Shelton, 1995; Wang *et al*. 1996). Kondo *et al*. (2004) showed that 2.3% condensed tannin in the ration containing tea waste had no negative effect on dry matter and NDF digestibility in goats. In the study by Harrison *et al*. (1973), the sheep fed by dry sainfoin had more necessary amino acid flow to the duodenum compared with the sheep which used alfalfa hay. Complexes of tannin-protein which are formed in the gastrointestinal tract are detectable in the form of fecal lignin which leads to negative apparent digestibility of lignin in the sheep fed by tannin-containing legumes (Silanikove *et al*. 1994) or negative digestibility of NDF (Silanikove *et al*. 1996) and ADF (Ben Salem *et al*. 2000). Based on the theory of Titgemeyer (1991), degradability of cell walls depended more on their structure than the environment or period of rumen retention. Low digestibility of cell wall not only limited energy consumption but also reduced feed consumption. Mokhtarpour (2012) reported that replacing 15% of pistachio by-product in the ration of dairy cattle did not have any effect on protein digestibility and organic matters of NDF and ADF. In this research, the nature of tannin and its low level caused tannin to have no negative effect on nutrient digestibility.

**Milk production and composition**

Woodward *et al*. (1999) reported that milk production of Friesian cows fed by *Lotus corniculantus* was 19% more than the cows fed by *Lotus corniculantus* +PEG. On the other hand, milk production of cows fed *Lotus corniculantus* was 60% more than those fed P. ryegrass. Considering

the different nature of tannins in two types of legumes and response to PEG, half of the lotus effect could be explained by the role of condensed tannins in this plant. In general, little research has been done on the role of tannins in milk production.

Bahatta *et al*. (2000) utilized the ration containing zero, 0.2% and 0.74% tannin in dry matter for feeding dairy cows using a type of Indian shell seed (*Tamarind usindica*). The results showed that the tannins in the ration not only had no negative effect on milk composition and production but also significantly increased milk protein ($P<0.05$). These researchers suggested that tannin in rations caused increase of amino acids flow to duodenal and, as a result, increased milk protein production.

Since the composition of tannin-protein in the rumen is stable and when entering an acidic environment, like the abomasum, the composition will break, increase of milk protein percentage is probably due to efficiency of feed protein use and increased essential amino acid flow to the small intestine.

Woodward *et al*. (1999) did not observe any difference in milk fat of Holstein cows fed by feed containing tannin compared with the treatment with PEG (a tannin inhibitor).

Generally, the results showed that processing protein supplements with tannin had no significant effects on the production and composition of cow's milk but caused a numerically increase in the production of milk and milk protein percentage of cows.

Tannins reduce protein degradability in the rumen and as a result, decrease production level of ammonia in the rumen (Ben Salem *et al*. 2003). In most experiments, tannins have reduced ammonia nitrogen in the rumen. Mokhtarpour (2012) reported that adding poly ethylene glycol (PEG) and urea to silage of pistachio by-product significantly increased ammonia nitrogen in the rumen.

This researcher found binding of tannins with poly ethylene glycol and urea and as a result, increases in protein degradability in the rumen was the reason for increases in ammonia nitrogen.

West *et al*. (1993) showed that, with the increase in the consumption of level of peanut skins in the rations of lactating Holstein cows, the level of rumen ammonia nitrogen was linearly reduced ($P<0.01$) and this phenomenon was attributed to the formation of tannin-protein complex that caused decreases in protein degradability in the rumen and resulted in the reduction of ammonia nitrogen concentration. Vahmani (2005) reported that adding 6% pistachio by-product to the ration of lactating Holstein cows did not have a significant effect on rumen ammonia nitrogen and concluded that low concentrations of tannins in the ration was the reason for this effect.

Norton *et al*. (1997) suggested that, besides reduction in available protein, reduction in the concentration of ammonia in rumen might also be generated by inhibiting proteolytic enzymes. Tannins also can reduce ammonia concentrations in rumen through inhibiting enzymatic activity of microbial deaminase and inhibiting enzymatic activity of urease. In this experiment, although there was no significant difference between soybean meal and processed soybean meal with tannin, tannins caused a decrease in rumen ammonia nitrogen; but, treatment of processed canola with tannin which contained the highest tannin concentration caused decreases in ammonia nitrogen of the rumen significantly. This reduction was probably due to the significant reduction in the production of ammonia nitrogen because of increase in the level of tannin and a reduction of protein degradability in the rumen.

In most experiment, tannins at low and moderate levels had no significant effect on pH of rumen. Concentrations less than 50 g per kg of dry mater of condensed tannin (5% of dry matter) in the ration had no significant effect on rumen fermentation (Barry and McNabb, 1999). Vahmani (2005) observed no significant difference in pH of rumen fluid in cows fed by 6% pistachio by-product compared with the control ration.

In this experiment, considering that processed treatments with tannins had the lowest pH of the rumen, probably tannins inhibited protein degradation in the rumen; as a result, the ammonia concentration was reduced and caused a reduction of pH in the rumen. Decreased in protein degradation in the rumen corresponded to the results of ammonia nitrogen produced in the rumen.

Although blood urea nitrogen was not affected by the treatments ($P>0.05$), processing the rations with tannin reduced blood urea nitrogen linearly (Table 4). The results of some studies on peanut skin have shown that increasing level of these by-products in rations of cows significantly reduced blood urea nitrogen ($P<0.05$) (Hill *et al*. 1986; West *et al*. 1993). These researchers suggested that, by increasing the concentration of ammonia in the rumen, due to the tannin in peanut skin, the ammonia entering the blood for conversion to urea was reduced. Vahmani (2005) and Bohluli (2007) also reported a non-significant decrease in blood urea nitrogen when using 6% and 15% of dry pistachio shell byproducts in a ration.

Research has shown that concentration of blood urea nitrogen, rumen ammonia and excreted nitrogen is less in ruminants fed legumes containing tannin than animals fed tannin-free forages. The results of these experiments correspond to most of the existing results; reduction of ammonia in rumen fed by processed rations with tannin is the reason for reduction in blood urea nitrogen.

## CONCLUSION

Results of this study show that under our current experimental conditions, processing protein supplements with tannin had no negative effects on animals. Further experiments should be done in this regard and on the rations containing higher levels of tannin.

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