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Effect of Tannin Extract from Pistachio by Product on *in vitro* Gas Production

Research Article

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ABSTRACT

This study was carried out to investigate the effects of treated protein supplements with tannin extracted from pistachio by-product (P-PB) on *in vitro* gas production using fistulated sheep. One portion of P-PB was mixed with four portions of water for 48 h. The extracted product was sprayed on soybean meal (SBM) or canola meal (CM) with an equal ratio (1:1 v/w) and dried in the shade. The experimental treatments included in this study were: 1) untreated soybean meal (USB), 2) soybean meal treated with tannin extract (SBTT), 3) untreated canola meal (UCM) and 4) canola meal treated with tannin extract (CMTT). Kinetics of gas production was fitted to an exponential model. After 96 h of incubation, the medium size of each syringe was used for determining ammonia N (NH3-N) concentration using distillation method. The results obtained from this study showed that spraying tannin extract on protein supplement increased the amount of tannin to 4.4, 3.13 in CM or SBM, respectively. Although gas production rate, fraction *b* and fraction *c* decreased by treated protein supplements in comparison with untreated protein supplements, the effects were not significant (P>0.05). The effect of tannin extract on NH3-N was significant (P≤0.05). The highest and the lowest content of NH_3 were for SBM with the lowest and CMTT with the highest content of tannin, respectively. Tannin from P-PB decreased organic matter digestibility (OMD), metabolizable energy (ME) and short chain fatty acid (SCFA) concentrations in treated protein supplements (P≤0.05). Untreated soybean meal and CMTT had the highest and the lowest content of OMD, ME and SCFA, respectively.

KEY WORDS gas production, metabolizable energy, pistachio by-product, protein supplements, tannin.

INTRODUCTION

Pistachio By-product (P-PB) P-PB is a high tannin feed which is produced in large amount (approximately 450000 tons per year) in many parts of Iran. The preliminary limitation of using this by product as a ruminant feed is the presence of high tannin content. Tannins are phenolic secondary compounds of plants that are usually classified to two groups of hydrolysable tannin (HT) and condensed tannin (CT) (Makkar, 2003). HT molecule contains a carbohydrate (generally, D-glucose) as a central core which is esterified with Gallic or Ellagic acid (Haslam, 1989). HT is mainly found in fruits, pods and plant galls (Mc Leod, 1974). CTs are oligomers of flavonoid units linked by carbon-carbon bonds (Hagerman, 1988). CTs are found in forage legumes, trees and shrubs. Tannins have both negative and positive effects on the ruminant. Using tannin at a level above 6% in ruminant rations mainly has adverse effects on the animal performance including feed intake, protein and fiber digestibility. The presence of tannins has been associated with lower nutritive values and lower biological availability of macromolecules like proteins and carbohydrates. However, low to moderate concentrations of tannins may improve digestive utilization of feed, mainly due to the reduc-

tion in protein degradation in the rumen and the subsequent increase in amino acid flow to the small intestine (Makkar *et al*. 2003), enhancing both efficiency of microbial protein synthesis and body protein retention. Effect on urea cycle and prevention from bloating in ruminant. In the presence of tannins in the rumen, plant proteins may bound to tannins and are protected from microbial degradation; but, the protein-tannin complex is released in the abomasum which in turn could lead to increased absorption of amino acids in the small intestine (Barry and Manley, 1984; Barry and Mc Nabb, 1999). Therefore, tannins may be either beneficial or harmful to animals depending on the type of consumed tannin, its chemical structure and molecular weight, ingested amount and the involved animal species. Gas production is an *in vitro* method for indirect measurement of substrate degradation by the rumen (Liu *et al*. 2002). This method is more efficient than the *in sacco* method in evaluating the effects of tannins (El-Waziry *et al*. 2007). 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 SBM or CM have been increasingly used as feed ingredients for ruminants. Therefore, the aim of this study was to determine the effects of treating SBM and CM with tannin (extracted by water from P-PB) on *in vitro* gas production.

MATERIALS AND METHODS

Sample preparation

One portion of fresh P-PB (containing soft hulls, twigs, small amounts of hard shells and green kernels from leaves) was mixed with four portions of water for 48 h. The extracted product was sprayed on SBM and CM with an equal ratio (1:1 v/w) and dried in the shade. The quantity of tannin and phenolic compounds was measured as described by Makkar (2003). The experimental samples were prepared by milling through a 2 mm mesh (SBM containing 938 g/kg DM, 470 g/kg crude protein and 121 g/kg ash and CM containing 917 g/kg DM, 352 g/kg crude protein and 133 g/kg ash).

Tannins were analyzed by first weighing 100 mg of each treatment into a 10 mL test tube. The samples were extracted using 70 aqueous acetone in an ultrasonic bath for 2 h and the contents were centrifuged for 20 min at 5000 × g and the supernatant was collected for tannin analyses. Each treatment contained three replicates and two blank samples. For the measurement of total phenolic compounds (TPC), 0.02 mL of acetone was extracted into a test tube and 0.480 mL distilled water was added and vortexed. Then, 0.250 mL Folin-Ciocalteau and 1.25 mL carbonate sodium were added and vortexed for 3-4 min. In the next step, the samples were kept at room temperature for 40 min.

Wavelengths reading was done in 725 nm by a spectrophotometer. In the final step, solution tannic acid was used, the standard curve was plotted and the amount of TPC was calculated (Makkar, 2003).

For the condensed tannin (CT) fraction, the extract was treated with Butanol-HCL in the presence of ferric ammonium sulphate and CT was expressed as leucocyanidin equivalent as follows:

CT= (A550 nm×782.6) / (Weight of sample dry matter)

Where:
A550 nm: is absorbance at 550 nm.

Assuming that effective E1 cm, 550 nm of leucocyanidin is 460) (Porter *et al*. 1986).

Insoluble polyvinyl polyprolidone (100 mg) was weighed into 100 mm × 12 mm test tubes. Distilled water (1 mL) and 1 mL of tannin containing the extract were added and vortexed. The tube was kept at 4 ˚C for 15 min, vortexed again and then centrifuged (3000×g) for 10 min. The supernatant was measured by Folin-Ciocalteau reaction and this was regarded as the non tannin phenol (NTP).

Total tannin phenol (TTP) was calculated as the difference of TPC and NTP.

The experimental treatments included with the study were: 1) untreated SBM (USB), 2) SBM treated with tannin extract (SBTT), 3) untreated CM (UCM) and 40 CM treated with tannin extract (CMTT).

Gas production

Gas production kinetics for the treatments was determined as described by Menke and Steingass (1988). Rumen fluid was supplied from three fistulated sheep fed twice per day with a diet containing Lucerne hay (600 g/kg) plus a concentrate mixture (400 g/kg) prior to the morning feeding. Rumen fluid was homogenized in a laboratory blender, filtered through three layers of cheese-cloth and purged by CO_2.

The temperature of the rumen fluid was maintained at 37-39 ˚C and mixed (1:2 v/v) with an anaerobic mineral buffer solution according to the procedure described by Makkar and Blummel (1995).

Preparation of *in vitro* mineral buffer media was as described by Menke and Steingass (1988). Buffer media per liter contained $NaHCO_3$, 7.00 g, NH_4HCO_3, 4.00 g, Na_2HPOo_4, 5.70 g, KH_2PO_4, 6.20 g, $MgSO_4$ $7H_2O$ 0.6 g, Na_2S, 0.52 g, $CaCl_2$ H_2O, 13.2 g, $MnCl_2$ $4H_2O$, 10.00 g, $CoCl_2$ 6H2o, 1.00 g, sodium resazurine, 0.01 g and 60 mL freshly prepared reduction solution containing 50 mg Na_2S $9H_2O$ and 3.7 Ml 1m NaOH. For the *in vitro* gas production experiment, 200 mg experimental sample was incu-

bated with 35 mL buffered rumen fluid containing CO_2 reflux in 100ml calibrated glass syringes fitted with plungers for 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h. Each treatment containing 4 replicates and 4 blank samples containing 30 ml of medium only were also included. The syringes were placed in Ben murry bath (39 ˚C) and were rotated during the experiment. Total gas production values were corrected for the blank. The OMD and ME of the samples were calculated using the equation given by Menke and Steingass (1988). Chain SCFA was calculated by the equation of Getachew *et al.* (1999). The estimated parameters were calculated by the following equations:

OMD (g/kg OM)= 148.8 + 8.89 GP + 4.5 CP + 0.651 XA
ME (MJ/kg DM)= 2.20 + 0.136 GP + 0.057 CP + 0.0029 CP2
SCFA (µmol L-1)= 0.0239 GP -0.0601

Where:
CP and XA: are percentage of crude protein and ash, respectively.
GP: is net gas production (mL/200 mg DM) after 24 h incubation.

Statistical analyses

After 96 h of incubation, the medium of each syringe was used for determining ammonia-N (NH3-N) concentration using distillation method (Kjeltec 1030 Analyzer Tecator, Hoganas, Sweden). Cumulative gas production data were fitted to the following exponential equation:

$y= b (1^{-e-ct})$

Where:
b: gas production from the insoluble fraction.
c: constant of gas production rate for the insoluble fraction (b).
t: incubation time.
y: gas produced at time "t".

Data of *in vitro* gas production and estimated parameters were subjected to analysis as a completely randomized design using the General Linear Model (GLM). Duncan's multiple range tests were used to compare the treatment means at ($P \leq 0.05$).

RESULTS AND DISCUSSION

The amount of tannin

The amounts of total phenolic compounds and total tannin phenols in treated and untreated protein supplements are shown in Table 1.

There are various results obtained from the amount of compound phenolic). Labavitch *et al.* (1982) reported that the amount of total phenolic compound (TPC) in maturity periods varied between 5.3 and 7.4% DM.

Table 1 The amount of total phenolic compounds and total tannin phenols in treated and untreated protein supplements

Treatments	TCP	TTP	CT
P-PB	9.93	5.73	1.47
USB	1.68	1.19	0.73
SBTT	5.61	4.30	0.82
UCM	3.01	2.34	0.68
CMTT	7.29	6.74	0.79

P-PB: pistachio by product; USB: untreated soybean meal; SBTT: soybean meal treated with tannin extract; UCM: untreated canola meal and CMTT: canola meal treated with tannin extract;
TPC: total phenolic compounds, TTP: total tannin phenols and CT: condensed tannin.

Bagheripour *et al.* (2008) showed that the amount of TPC, total tannin phenols (TTP) and CT from P-PB was 14.11, 9.71 and 0.91, respectively. Similar to the present study, Mokhtarpour (2012) reported that TPC, TT, CT from PB was 10.37, 6.44, and 1.27%, respectively. This inconsistency between different studies might be due to varieties, maturity age and methods of drying systems. Spraying tannin extract on protein supplements increased amount of tannin to 4.4, 3.13 in CM and SBM, respectively. CMTT and USB had the highest and the lowest amount of tannin, respectively. Tannins are one of compounds within CM and their presence makes a dark in CM. 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.

Gas production and estimated parameters

Curve of cumulative gas production is shown in Figure 1.

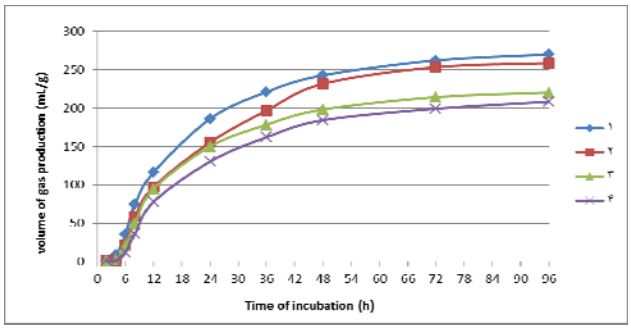

Figure 1 Relationship between cumulative gas production and time of incubation
1) Untreated soybean meal 2) soybean meal treated with tannin extract 3) untreated canola meal and 4) canola meal treated with tannin extract

The protein supplements treated with tannin extract decreased gas production values 24 h after incubation.

Although tannins did not significantly affect fraction *b* and gas production values, depress by treated protein supplements in comparison with untreated protein supplements, fraction *c* had significantly decreased when soybean meal was treated with tannin extract (Table 2).

USB had the highest gas production value and CMTT had the lowest gas production value, fraction *b* and fraction *c* (Table 2). Tannins can decrease cumulative gas production by forming tannin-macromolecule complex which inhibits microbial enzyme activities (Mcsweeny *et al*. 2001; Tabacco *et al*. (2006) showed that high tannin concentration in the diet may be a cause for reduction in microbial enzyme activities like cellulase.

Bento *et al*. (2005) reported that mimosa tannin depressed gas production rate and concluded that this reduction might bind tannin with microorganisms or their enzymes. El-Waziry *et al*. (2005) and Mohammadabadi *et al*. (2010) reported that adding tannic acid (hydrosable tannin) to SBM decreased gas production cumulative and fraction *b* and *c* and concluded that processing SBM with tannic acid protected protein from degradation in the rumen. The mean values of NH3-N concentrations are shown in Table 2. The effect of tannin extract on NH3-N was significant (P≤0.05). Content of NH3 was highest for SBM which had the lowest tannin content and was the lowest for CMTT which had the highest tannin content.

Table 2 Gas production parameters and NH3 of protein supplements treated with tannin extract from pistachio by-product

Treatments	*b* (mL)	*C* (mL h^{-1})	OMD (g kg)	ME (MJ kg^{-1})	SCFA (μmol L^{-1})	NH3 (mg/dL)
USB	309.3±4.86	0.0546±0.0026	482.02±4.06^{a}	7.27±.06^{a}	0.825±0.011^{a}	34.2±1.12^{a}
SBTT	302.3±8.87	0.0432±0.0042	427.99±6.65^{b}	6.44±.09^{b}	0.677±0.017^{b}	32.8±0.78^{a}
UCM	258.1±4.73	0.0526±0.003	417.182±2.3^{b}	6.29±.03^{c}	0.65±0.006^{b}	32.1±0.94^{ab}
CMTT	244.6±3.73	0.045±0.0022	382.98±2.56^{c}	5.75±.03^{d}	0.55±0.007^{c}	29.4±0.35^{c}

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
USB: untreated soybean meal; SBTT: soybean meal treated with tannin extract; UCM: untreated canola meal; CMTT: canola meal treated with tannin extract; *b*: gas production from fermentable fraction; *c*: constant of gas production rate; OMD: organic matter digestibility; ME: metbolizable energy; SCFA: short chain fatty acid, NH3: concentration of ammonia N.

This result was in agreement with the result obtained by Sliwinski *et al*. (2002) and El-Waziry *et al*. (2005) who reported reducing ruminal NH3-N concentration by tannins. Moreover, Mohammadabadi and Chaji *et al*. (2012) demonstrated significant reduction in NH3-N content while adding oak fruit tannin, pistachio hull tannin and pistachio leave tannin to SBM.

Lower ammonia concentrations were mainly due to the reduction in amino acids degradation in the rumen (Frutos *et al*. 2004). Tannins have been shown to protect dietary protein from ruminal degradation and could be used advantageously to increase bypass protein and to improve ruminant performance (Makkar, 2003). According to this research, with increasing the amount of tannin content, degradation of protein reduced in the resulted ammonia concentration was decreased.

The effects of tannins on OMD, ME and SCFA are shown in Table 4. Protein supplements treated with tannin extracted from PB had a significant effect on OMD, ME and SCFA, USB had the highest and CMTT had the lowest OMD, ME and SCFA. Mohammadabadi *et al*. (2009) showed that SBM treated with tannin of oak leave decreased OMD and ME. Tabacco *et al*. (2006), also reported that OMD decreased by about 5.1% with tannic acid. Mohammadabadi *et al*. (2010) reported that adding tannic acid to sunflower meal decreased OMD, ME and SCFA. The correlation between gas production and SCFA was positive (Menke and Steingass, 1988).

The SCFA contributed by at least 65 to 75% of the total metabolizable energy supply (Penner *et al*. 2009). Gas volumes were produced quantitatively and qualitatively as a result of SCFA production. The decrease of OMD and ME in this study was probably due to the formation of complex between tannins and macromolecules, like proteins and carbohydrates, which caused reduction in the activities of rumen microbial proteolytic and cellulolytic enzymes; as a result, decrease in the degradability of these macromolecules happened.

CONCLUSION

In this study, although protein supplements treated with tannin extract from PB did not significantly affect degradation, gas production, OMD, ME and SCFA, they were linearly decreased. Spraying tannin extract on protein supplement increased the amount of tannin to 4.4, 3.13 in CM or SBM. Although gas production rate, fraction *b* and fraction *c* decreased by treated protein supplement in comparison with untreated protein supplement, this decrease was not significant. Protein supplements treated with tannin extract significantly decreased N-NH3. So, treatment 4 had the lowest and treatment 1 had the highest amount of N-NH3. Thus, the supplements treated with tannin PB decreased degradation of protein. It seems that protein supplements treated with water solution of tannin extracted from pistachio by product can improve nutritive value of protein supplements.

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