The effects of dried pistachio (Pistachio vera L.) by-product addition on corn silage fermentation and in vitro methane production

Nihat Denek*a, Sadik Serkan Aydinda and Abdullah Canb

aFaculty of Veterinary, Department of Animal Science, Harran University, Sanliurfa, Turkey; bSanliurfa Food Control Laboratory, Ministry of Food, Agriculture and Livestock, Sanliurfa, Turkey; cFaculty of Agriculture, Department of Animal Science, Harran University, Sanliurfa, Turkey

ABSTRACT

The objective of this study was to determine the effect of adding different levels of pistachio by-product (PB) on the fermentation quality, nutritive value and in vitro methane (CH4) production of corn silage. For this purpose, silages were prepared in the 1.5 l glass jars by adding with 2%, 4%, 6%, 8% and 10% pistachio PB (as a dry matter basis). The results showed that addition of 6%, 8% and 10% pistachio PB levels decreased (P<0.05) silage acetic acid content. Increment of pistachio PB level increased (P<0.05) silage propionic acid content, but it decreased (P<0.05) ammonia nitrogen values. Addition of all levels of pistachio PB decreased (P<0.05) gas production (from 251.70 to 196.76 ml/g dry matter (DM)), in vitro organic matter digestibility (from 70.26% to 61.62%) and metabolizable energy (from 9.69% to 8.27 MJ/kg DM) values. Similarly, increment-level pistachio PB decreased (P<0.05) of in vitro methane production level. As a result, pistachio PB might have a potential usage value as silage additive when undesired ruminal methane production is aimed to be suppressed by ruminant.

1. Introduction

According to the 2012 data, pistachio (Pistachio vera L.) production of Turkey was 150,000 tonnes (Tuik 2013), and Turkey is among the top five pistachio-producing countries. Approximately 10–12 thousand tonnes of dry pistachio by-product (PB) was obtained from 25–30 thousand tonnes of wet pistachio PB from processing plants. Nutrient composition of pistachio PBs obtained following pistachio processing is affected by its variety, harvest season, processing methods, as well as leaf and twig contents (Bagheripour et al. 2008; Valizadeh et al. 2009; Gholizadeh et al. 2010; Mokhtarpour et al. 2012; Boga et al. 2013). According to former studies, wet pistachio by-product can be ensiled alone or in the combination with different feed sources, and these silages can be used as animal feeds because of its high quality (Vahmani et al. 2007; Valizadeh et al. 2009; Mokhtarpour et al. 2012). Depending on the condensed tannin content, pistachio PB might be used in animal nutrition and its usage prevents environmental pollution and provides added value to economy.

To our knowledge, we did not come across any work on the addition of dried pistachio by-product (DPB) to corn silage material before ensiling during literature search in this study. Only works on addition of DPB into total mixed ration or on ensiling of sole wet pistachio by-products are available in the literature. Shakeri et al. (2014) ensiled wet pistachio by-product without any silage additive and reported that pistachio PB silage had been able to carry criterions of high-quality silages. Ammonia-N (NH3-N) values also decreased with molasses addition as well. The tannin content of pistachio PB depends on factors such as climate, pistachio variety and rainfall amount (Bohluli et al. 2007; Hashami et al. 2008). Type, molecular weight and the ingested amount of tannin may have a positive or negative effect on ruminant animals (Frutos et al. 2004). Former studies stated that tannins suppress number of cellulolytic bacteria and ruminal methane production (Newbold et al. 1997; Min et al. 2003; Bhatta et al. 2009; Goel & Makkar 2012). The condensed tannin content at the level of 2–3% of diet can decrease protein degradation in the rumen and depending on the solubility of tannin–protein complex in the medium of abomasum and its lower pH (<3.5) is reported to enhance the intestinal absorption of essential amino acids (Jones & Mangan 1977; Frutos et al. 2004). In case of usage of more than 5% condensed tannin in dry matter content of the grain, proteins are excreted in the faeces without being absorbed in the intestines due to the formation of excessive levels of tannin–protein complex; therefore it could reduce the protein utilization of ruminant animals (Kumar & Singh 1984). Ensiling of high tannin-containing feed materials can lower tannin content as a result of oxidation of phenolic compounds during ensiling process (Ben Salem et al. 2005). Usage of 2.0–4.5% of condensed tannin in the ration dry matter increased milk production (Wang et al. 1996) and milk protein levels (Bhatta et al. 2000). This study was carried out to use of DPB as a silage additive at different levels to corn silage and determine its effects on in vitro methane production and silage quality values.

2. Material and methods

Corn silage material was provided from Harran University Animal Research Field and it was harvested at dough stage.
Pistachio (*Pistacia vera* L.) PB was obtained from the private pistachio-processing facility in Sanliurfa and it was dried at room temperature to use as a silage additive. For this purpose, experimental silage treatments consisted of five replicates for each treatment, including control corn silage and adding 2%, 4%, 6%, 8% and 10% pistachio PB (as a dry matter basis) to corn silage. Silages were prepared in the 1.5 L glass jars. The jars were stored for 60 days at room temperature and were opened after 60 days of ensiling.

The pH values and dry matter contents of the silages were immediately measured. A total of 25 g of fresh silage macerated with 100 ml distilled water with a high-speed blender. The macerated silage samples were filtered through two layers of cheesecloth and the pH values of the filtrate were measured with a laboratory pH meter (WTW-7310) (Polan et al. 1998). After pH determined, 10 ml filtrate was acidified with 0.1 ml 1 M HCl (v/v) and stored at −18°C for NH₃-N analysis. The NH₃-N content was analysed according to Broderick and Kang (1980) by the Kjeldahl method. Other 10 ml filtrate was acidified with (0.25 ml of 25%) metaphosphoric acid. Volatile fatty acids (VFA) and lactic acid were determined by high-performance liquid chromatography according to the method reported by Suzuki and Lund (1980).

Dry matter contents of the silages were determined by drying 20 g of the ensiled forage at 60°C for 48–72 h in a forced-air oven and then weighing them. After weighing, the dried sample and pistachio PB were ground through 1-mm screen in a laboratory mill (Wiley mill) and analysed for crude protein (CP) content by the AOAC (2005) method. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) content was analysed according to methods described by Van Soest et al. (1991). The gas production values of the silages, pistachio PB and corn herbage were determined through the method described by Menke and Steingass (1988) using four glass syringes as replicate. The in vitro organic matter digestibility (IVOMD) (g/kg OM) and metabolizable energy (ME) (MJ/kg DM) of silages were calculated using equations reported by Menke et al. (1979) as:

\[
\text{ME(MJ/kg DM)} = 2.20 + 0.136 \times \text{Gp} + 0.057 \times \text{CP} + 0.0029 \times \text{CP}^2, \\
\text{IVOMD(%) = 14.88 + 0.889 \times \text{Gp} + 0.45 \times \text{CP} + 0.0651 \times \text{XA},}
\]

where CP is CP in g/100 g DM, crude ash in g/100 g DM and gas production is the net gas production (ml) from 200 mg DM after 24 h of incubation.

After recording 24-h gas production values, gas inside the syringe was taken by three-way syringe system and total gas was injected into computer-assisted infrared methane gas meter (Sensor Europe GmbH, Erkrath, Germany) and then methane content was determined as a percentage of 24 h the total amount of gas formed (Goel et al. 2008). The statistical analysis of results included one-way analysis of variance using SPSS program (1991). Duncan multiple comparison test was used for comparison of group averages.

### 3. Results and discussion

Nutrient composition, condensed tannin content, methane production as percentage of total gas production, IVOMD and ME content of corn ensiling material and DPB are presented in Table 1. CP, ME value, and IVOMD of PB of pistachio varieties grown in Turkey were reported in study in a range of 7.27–14.99%, 9.76–11.05 MJ/kg DM and 69–72.65%, respectively (Boga et al. 2013). In the study of Forough and Fazaeli (2005), CP, NDF, ADF and ME values were determined as 9.2–12.0%, 30–36%, 21–28% and 7.1–7.5 MJ/kg DM, which were consistent with obtained values of this study. Condensed tannin content of pistachio PB in the present study (2.62%) was similar to the values (2.07–2.63%) reported by Boga et al. (2013), but it was higher than the value (1.81%) reported by Ghaffari et al. (2014). These differences can vary depending on variety of pistachio, geographic conditions, soluble sugar content of pistachio and harvest time (Bohluli et al. 2007).

The effects of adding different levels of DPB on nutrient composition of corn silages are shown in Table 2. In the present study, addition of 4% or more pistachio PB increased (P < 0.05) DM, crude ash and ADF contents of corn silages. Only addition of 8% and 10% pistachio PB increased (P < 0.05) CP contents of silages. Increment of DM content of silages can be explained with inhibition activity of butyric acid bacteria and many microorganisms depending on pH reduction in silo jars (Henderson et al. 1982) and in addition to higher DM content of pistachio PB. Similarly, homofermentative lactic acid bacteria produce lactic acid from the glucose, fructose and other sugars inside the silo and therefore loss of DM decreases depend on increment lactic acid content of silages (Kung & Shaver 2001). Addition of 8% and 10% pistachio PB also increased (P < 0.05) CP contents of silages. This can be explained with the formation of tannin–protein complexes, which can cause a reduction in the rate of microbial degradation of proteins (Santos et al. 2000), and relatively higher CP content of pistachio PBs than corn silage material. Pistachio PB increased (P < 0.05) ADF values of silages depending on the increment level in the present study. Bacillus group fungi and some bacteria inside the silo are able to produce the cellulase enzyme (Tomme et al. 1995). In our study, high level of ADF depending on the pistachio PB increment in silages can be explained due to antimicrobial effect of tannin, which causes reduction of cellulase enzyme production and activity (Makkar et al. 1987). In contrast, 2% and 10% of pistachio PB levels decreased NDF values of silages. It can be concluded that the effect of tannin on hemicellulose may not be the same as that on cellulose.

The pH, organic acids (acetic acid, propionic acid, lactic and butyric acid) and ammonia nitrogen values of silages are presented in Table 3. All the DPB added silages in this study had pH values in range of 3.85–3.93, which are the embraced by values (3.8–4.2) of good-quality silages formerly stated (Leterme et al. 1992). Tannins and phenolic monomers possess antimicrobial activity on both cellulolytic and proteolytic bacteria (Goel et al. 2005). Break down of protein, organic acids and carbohydrates by proteolytic bacteria inside the silo causes acetic acid production (Kung & Shaver 2001). In this study, addition of more than 6% pistachio PB to corn ensiling material reduced the acetic acid values. This result occurred due to antimicrobial activity on proteolytic bacteria and protein-binding effect of tannin included in pistachio PB. In former studies, addition of tannin-containing
Table 1. Chemical composition, condensed tannin content, methane production, IVOMD and ME of corn ensiling material and DPB.

| Chemical composition | Control | 2% DPB | 4% DPB | 6% DPB | 8% DPB | 10% DPB | SEM |
|----------------------|---------|--------|--------|--------|--------|--------|-----|
| DM                  | 30.82   | 93.40  | 93.40  | 93.40  | 93.40  | 93.40  | 0.182|
| Ash                 | 5.93    | 15.79  | 15.79  | 15.79  | 15.79  | 15.79  | 0.011|
| CP                  | 8.22    | 9.43   | 9.43   | 9.43   | 9.43   | 9.43   | 0.019|
| NDF                 | 61.85   | 29.19  | 29.19  | 29.19  | 29.19  | 29.19  | 0.035|
| ADF                 | 34.18   | 23.15  | 23.15  | 23.15  | 23.15  | 23.15  | 0.072|
| CT                  | 2.29    | 26.17  | 26.17  | 26.17  | 26.17  | 26.17  | 0.037|
| CH₄                 | 12.51   | 1.77   | 1.77   | 1.77   | 1.77   | 1.77   | 0.037|
| IVOMD               | 68.40   | 61.79  | 61.79  | 61.79  | 61.79  | 61.79  | 0.035|
| ME                  | 8.92    | 7.11   | 7.11   | 7.11   | 7.11   | 7.11   | 0.035|

Notes: CM: corn ensiling material; DPB: dried pistachio by-product; DM: dry matter; % Ash: crude ash; % CP: crude protein; % NDF: neutral detergent fiber; % ADF: acid detergent fiber; % CT: condensed tannin; g/kg DM; CH₄: methane, as a percentage of total gas production; % IVOMD: in vitro organic matter digestibility, % ME: metabolisable energy, MJ/kg DM.

Gleditsia triacanthos fruit and pomegranate pulp to ensiling material reduced acetic acid content but they increased propionic acid content (Canbolat, Kalkan, et al. 2013, Canbolat, Kamalak, et al. 2014). Our results are in agreement with those former studies. All the DPB added silages in this study had organic acid values in a range of good-quality silages in which lactic acid, acetic acid, propionic acid, and butyric acids have 4–7%, 1–3%, <0.1% and 0% values, respectively (Kung 2008). Addition of 2% and 8% pistachio PB reduced the pH of silages compared to control corn silage (P < 0.05). Pistachio PB did not affect lactic acid content of silages. Whilst increment of pistachio PB in silages reduced acetic acid values from 16.21 to 13.16 g/kg DM and propionic acid values increased from 0.34 to 0.41 g/kg DM. Addition different levels of pistachio PB lowered NH₃-N values of silages (< 0.05). Increment of pistachio PB in silages lowered NH₃-N values from 3.36 to 1.69% of total silage N compared to control corn silages. Protein degradation inside the ensiling material are degraded highly due to increase in the number of enterobacter or clostridial bacteria varieties (Kumar & Singh 1984). Tannins in silos, in an environment close to a neutral pH, form complex compounds with soluble protein silage, thereby preventing the degradation of proteins by microorganisms and the subsequent reduction of the ammonia nitrogen concentration. This process prevents protein losses and improves silage quality (Barry & Manley1966; Santos et al. 2000).

The effects of adding different levels of DPB on in vitro methane production and digestibility of silages are presented in Table 2. The effects of adding different levels of DPB on in vitro gas and methane production. Methane contents in total produced gas and ME values of silages compared to control corn silage (P < 0.05). Increment of pistachio PB in the corn silages lowered in vitro total gas production, methane production, IVOMD and ME values of silages compared to control corn silage in this study (P < 0.05). Pistachio PB used as a feed additive in in vivo (Mokhtarpour et al. 2012) and in vitro (Bagheripour et al. 2008) studies reduced gas production values. The result of this study was in agreement with the results of those studies. Microbial degradation of carbohydrates was reduced in tannin-containing feedstuffs because of binding of microorganisms or enzymes to tannin (Muhammad et al. 1994) or diminishing number of bacteria bond to feed particles decreased gas production values previously reported (Alipour & Rouzbehian 2010). Babayemi et al. (2004) also reported that varying in vitro gas production values may be associated with condense

Table 3. Effect of different levels of DPB on pH, organic acids and ammonia nitrogen content of silages.

| Item   | Control | 2% DPB | 4% DPB | 6% DPB | 8% DPB | 10% DPB | SEM |
|--------|---------|--------|--------|--------|--------|--------|-----|
| pH     | 3.89    | 3.85   | 3.92   | 3.93   | 3.86   | 3.91   | 0.011|
| AA     | 16.21   | 15.76  | 14.91  | 13.92  | 13.16  | 14.26  | 0.519|
| PA     | 0.34    | 0.47   | 0.44   | 0.40   | 0.42   | 0.41   | 0.01 |
| LA     | 69.70   | 70.81  | 70.89  | 69.43  | 70.21  | 69.12  | 0.704|
| BA     | ND      | ND     | ND     | ND     | ND     | ND     | 0.09 |
| NH₃-N  | 3.36    | 3.03   | 2.44   | 1.82   | 1.84   | 1.69   | 0.09 |

Notes: Values in the same row without a common superscript letter are significantly different (P < 0.05). DPB: dried pistachio by-product; pH: pH value; AA: acetic acid, g/kg DM; PA: propionic acid, g/kg DM; ND: not detected; LA: lactic acid, g/kg KM; BA: butyric acid, g/kg KM; NH₃-N: ammonia nitrogen, NH₃-N/TN.

Table 4. Effect of different levels of DPB on in vitro gas and methane production.

| Item   | Control | 2% DPB | 4% DPB | 6% DPB | 8% DPB | 10% DPB | SEM |
|--------|---------|--------|--------|--------|--------|--------|-----|
| GP     | 251.70  | 225.26 | 203.95 | 227.40 | 211.67 | 196.76 | 5.550|
| CH₄    | 12.11   | 10.95  | 10.96  | 10.32  | 9.82   | 8.44   | 0.169|
| IVOMD  | 70.28   | 65.88  | 62.15  | 66.49  | 64.72  | 61.62  | 0.957|
| ME     | 9.69    | 9.01   | 8.41   | 9.07   | 8.68   | 8.27   | 0.149|

Notes: Values in the same row without a common superscript letter are significantly different (P < 0.05). DPB: dried pistachio by-product; GP: 24 h gas production ml/g DM; CH₄: methane production as a percentage of total gas production, %; IVOMD: in vitro organic matter digestibility, %; ME: metabolizable energy, MJ/kg DM.
tannin, CP and cell wall content of pistachio PB in their study. In a study, replacing partially alfalfa hay with pistachio PB in sheep diets diminished number of total and cellullosic bacteria number (Ghasemi et al. 2012). It reported that reduction of methane production was related with suppressing effect of tannin on protozoa (Moss et al. 2000). Tannins decrease acetic acid production in the rumen via their negative effects on cellullosic bacteria. Tavendale et al. (2005) explained methane reduction via reduction in fibre digestion, which decreases H₂ production, and via inhibition of the growth of methanogens. Lower proportion of acetate and simultaneous higher proportion of propionate also contributes to reduction of methane at higher levels of tannin inclusion. Acetate formation from pyruvate in the rumen produces metabolic hydrogen, which is a main precursor of methanogenesis; in contrast, propionate formation from pyruvate requires hydrogen (Moss et al. 2000). Role of condensed tannins in decreasing methane production with a depression of fibre digestion via formation of hydrogen bonds between free phenolic functional groups and the fibre (Silanikove et al. 2001). IVMOD’s Ghadi, Kaleghuchi, white varieties of pistachio PB were determined as 67.4%, 71.9% and 70.9%, respectively (Bohluli et al. 2007). These values are higher than those (61.79%) reported in this study. However, digestibility values (52.6–65.6%) reported by Noghabi and Rouzbahian (2011) are in an agreement with those reported in the present study. Only or partially ensiling pistachio PB containing feedstuffs had lower in vitro gas production, organic matter digestibility and ME values in some studies (Bagheripour et al. 2008; Mohammadbadi & Chaji 2012), which are consistent with the result of this present study.

4. Conclusion

As a result, DPB can be used as feed when it is added to corn plant ensiling materials. In this way, this by-product may have economic merit in addition to reducing undesired ruminal methane production. However, these results should be supported with in vivo feeding studies in future.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Alipour D, Rouzbahian Y. 2010. Effects of several levels of extracted tannin from grape pomace on intestinal digestibility of soybean meal. Livestock Sci. 128:87–91.

AOAC. 2005. Official methods of analysis. 18th ed. Maryland, MD: AOAC International.

Babayemi OJ, Demeyer D, Fievez V. 2004. Nutritive value and qualitative assessment of secondary compounds in seeds of eight tropical browse, shrub and pulse legumes. Commun Agric Appil Biol Sci. 69:103–110.

Bagheripour E, Rouzbahian Y, Alipour D. 2008. Effects of ensiling, air-drying and addition of polyethylene glycol on in vitro gas production of pistachio by-products. Anim Feed Sci Technol. 146:327–336.

Barry TN, Manley TR. 1986. Interrelation ships between the concentrations of total condensed tannin, free condensed tannin and lignin in Lotus sp. and their possible consequences in ruminant nutrition. J Sci Food Agric. 37:248–254.

Ben Salem H, Makkar HPS, Nezafati A, Hassayoun L, Abidi S. 2005. Benefit from the association of small amounts of tannin-rich shrub foliage (Acacia cyanophylla Lindl.) with soybean meal given as supplements to Barbarine sheep fed on oat hay. Anim Feed Sci Technol. 122:173–186.

Bhatta R, Krishnamoorthy U, Mohammed F. 2000. Effect of feeding tamarind (Tamarindus indica) seed husk as a source of tannin on dry matter intake, digestibility of nutrients and production performance of crossbred dairy cows in mid-lactation. Anim Feed Sci Technol. 83:67–74.

Bhatta R, Uyeno Y, Tajima K, Takenaka Y, Yabumoto Y, Nonaka I, Enishi O, Kurihara M. 2009. Difference in the nature of tannins on in vitro ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. J Dairy Sci. 92:5512–5522.

Boga M, Guven I, Atalay Al, Kaya E. 2013. Effect of varieties on potential nutritive value of pistachio hull. Kafkas Univ Vet Med J. 19:699–703.

Bohluli A, Naserian A, Valizadeh R, Eftekharshahroodi F. 2007. The chemical composition and in vitro digestibility of pistachio by-product. Proceedings of British Society of Animal Science; p. 223. Scarborough, UK.

Broderick GA, Kang JH. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J Dairy Sci. 63:64–75.

Canbolat O, Kalkan H, Filya I. 2013. Yonca silajların katkı maddesi olarak gladciya meyveylerin (Gleditsia triacanthos) kullanımı olanakları. Kafkas Univ Vet Med J. 19:291–297.

Canbolat O, Kamalak A, Kara H. 2014. Nar posası silajina (Punica granatum L.) katılan ürenin silaj fermentasyonu, aerobic stabilitesi ve in vitro gaz üretimini üzerine etkisi. Ankara Univ Vet Fak Derg. 61:217–223.

Forough N, Fazaelli H. 2005. Studies on the different methods of ensiling pistachio by-products. In: Third seminar of animal nutrition. Karaj: Hidarabad Research Center.

Frutos P, Hervas G, Giraldez FJ, Mantecone AR. 2004. An in vitro study on the ability of polyethylene glycol to inhibit the effect of quebracho tannins and tannic acid on rumen fermentation in sheep, goats, and deer. Aust J Agric Res. 55:1125–1132.

Gaffar MR, Ta&Mabbi AM, Khorvash M, Naserian AA, Vakili AR. 2014. Effects of pistachio by-products in replacement of alfalfa hay on ruminal fermentation, blood metabolites, and milk fatty acid composition in Saanen dairy goats fed a diet containing fish oil. J Appl Anim Res. 42:186–193.

Ghasemi S, Naserian AA, Valizadeh R, Rahmasti AM, Vakili AR, Behgar M, Ghovvati S. 2012. Inclusion of pistachio hulls as a replacement for alfalfa hay in the diet of sheep causes a shift in the rumen cellulytic bacterial population. Small Rumin Res. 104:94–98.

Gholizadeh H, Naserian AA, Valizadeh R, Tahmasebi AM, Vakili AR, Behgar M, Ghovvati S. 2012. Inclusion of pistachio hulls as a replacement for alfalfa hay in the diet of sheep causes a shift in the rumen cellulytic bacterial population. Small Rumin Res. 104:94–98.

Gholizadeh H, Naserian AA, Valizadeh R, Tahmasebi A. 2010. Effect of feeding pistachio byproduct on performance and blood metabolites in Holstein dairy cows. Int J Agric Biol. 12:867–870.

Goel G, Makkar HPS, Becker K. 2008. Effect of Sesbania sesban and Carduus pycnocephalus leaves and Fenugreek (Trigonella foenum-graecum L.) seeds and their extract on partitioning of nutrients from roughage-and concentrate-based feeds to methane. Anim Feed Sci Technol. 147:72–89.

Goel G, Makkar HPS. 2012. Methane mitigation from ruminants using tannins and saponins, a status review. Trop Anim Health Prod. 44:729–739.

Goel G, Puniya AK, Singh K. 2005. Xylanolytic activity of ruminal Streptococcus bovis in presence of tannin acid. Annu Microbiol. 55:295–27; Charlotte, NC (USA).

Hashami O, Naserian A, Tahmasebi AM, Valizadeh R, Mohammadbadi T. 2008. Degradability of pistachio by-products at different harvesting times. In: Proceedings of British Society of Animal Science. Scarborough: BSAS; p. 251.

Henderson AR, McDonald P, Anderson D. 1982. The effect of a cellulase preparation derived from Tricoderma viride on the chemical changes during the ensilage of grass, lucerne and clover. J Sci Food Agric. 33:16–20.

Jones WT, Mangan JL. 1977. Complexes of the condensed tannins of sainfoin (Onobrychis vicifolia Stop) with fraction 1 leaf protein and with submaxillary mucopolysaccharide, and their reversal by polyethylene glycol and pH. J Sci Food Agric. 28:126–136.

Kumar R, Singh M. 1984. Tannins: their adverse role in ruminant nutrition. J Agric Food Chem. 32:447–453.

Kung JRL. 2008. Silage fermentation end products and microbial populations: their relationships to silage quality and animal productivity. Proc. 2008 Annual Conference of the American Association of Bovine Practitioners; Sept 25–27; Charlotte, NC (USA).
