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Short communication

# Nutritive value and polyphenol content of pomegranate seed pulp ensiled with different tannin-inactivating agents

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ABSTRACT

This study was conducted to investigate the effect of addition of polyethylene glycol (PEG), urea, and calcium hydroxide as tannin-inactivating agents, on the chemical composition, polyphenolic compounds content, and rumen degradability of ensiled pomegranate seed pulp (PSP). The experimental treatments were as: (1) PSP ensiled without additive, (2) PSP ensiled with PEG, (3) PSP ensiled with urea, and (4) PSP ensiled with calcium hydroxide. The results indicated that urea and calcium hydroxide addition reduced ($P < 0.001$) the total polyphenolic compounds (TP) and total tannin (TT) contents of PSP, whereas the condensed tannin (CT) content was not affected by the additives. The hydrolyzable tannin (HT) content of PSP was reduced ($P < 0.05$) with calcium hydroxide addition, and the levels of punicalagin A and ellagic acid (EA), which are potent antioxidants, were decreased ($P < 0.02$). PEG and calcium hydroxide additives decreased the rumen and total tract digestibility of the DM of PSP ($P < 0.006$ and $P < 0.02$, respectively). Urea appeared to be the most suitable chemical agent for ensiling PSP because it decreased the TP and TT contents of PSP, did not affect punicalagin A and EA content, and improved rumen and total tract digestibility of the DM of PSP.



## 1. Introduction

Pomegranate is an important source of bioactive compounds, and has been used in traditional medicine over centuries. The pomegranate seed pulp (PSP), a by-product of industrial extraction of pomegranate, contains large amounts of oil and other nutrients; however, it also contains some tannins that may have adverse effects on animal performance (Abbasi et al., 2008). Although tannins are able to bind to macromolecules and impair the degradation of these molecules and reduce animal's feed intake and/or even produce toxicosis (McSweeney et al., 2001; Silanikove et al., 2001), some polyphenolic compounds in tannins, mostly in hydrolysable tannins (HT), possess antioxidant activity that may improve health. Pomegranate fruit, including PSP, contains HT in the form of oligomeric ellagitannins (mainly punicalagins) (Clifford and Scalbert, 2000; Reed et al., 2005). These compounds account for much of pomegranate antioxidant activity and could act as an antioxidant (Negi and Jayaprakasha, 2003). Numerous studies have determined the effect of different chemical agents, including alkaline solutions, urea, and polyethylene glycol (PEG), on the inactivation of tannin in certain feeds (Murdiati et al., 1990; Ben Salem

*Abbreviations:* ADF, acid detergent fiber; CP, crude protein; CT, condensed tannins; DM, dry matter; EA, ellagic acid; HPLC, high-performance liquid chromatography; HT, hydrolysable tannins; GA, gallic acid; $NH_3$-N, ammonia nitrogen; PEG, polyethylene glycol; TMR, totally mixed ration; NDF, neutral detergent fiber; PSP, pomegranate seed pulp; TP, total polyphenolic compounds; TT, total tannins; WSC, water soluble carbohydrate.

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et al., 1999); however, to date, no study has been conducted to evaluate the impact of these chemicals on beneficial tannins such as those with antioxidant activity. Thus, the main objective of the present study was to determine the ability of various tannin-inactivating agents to reduce the tannin content of ensiled PSP, improve its DM digestibility, as well as retain its useful polyphenolic compounds content.

## 2. Materials and methods

The PSP (containing 475 g/kg DM) was ensiled in 3-L plastic buckets and sealed (with a density of 600 kg/m$^3$, so each bucket was contained about 1.8 kg of wet PSP) without any additive or with the addition of PEG, urea, and calcium hydroxide at a rate of 90, 20, and 20 g/kg DM of PSP, respectively (each with three replicates). The silos, which were stored at 25 °C, were opened after 60 days, and a PSP sample from each bucket was equalized for freezing at −20 °C, or drying at 60 °C for 48 h and subsequently milling by using a hammermill with 2-mm screen.

### 2.1. Chemical analysis

The dried feed samples were analyzed for crude protein (CP) expressed as $N \times 6.25$ (Kjeldahl method, Kjeltec 2300 Auto-analyzer, Foss Tecator AB, Hoganas, Sweden), acid detergent fiber ([ADF], Van Soest et al., 1991), neutral detergent fiber ([NDF], Van Soest et al., 1991), ether extract ([AOAC, 2000], ID 920.39), water soluble carbohydrate ([WSC], Dubois et al., 1956), and ash ([AOAC, 2000], ID 942.05) contents. Sodium sulfite and alpha amylase were not used in the NDF assay, and NDF was expressed as the ash-free residue after extraction with boiling neutral solutions of sodium lauryl sulfate and EDTA. The silage pH, lactic acid content, and ammonia nitrogen ($NH_3$-N) content were determined with water extracts of fresh preserved samples (Nishino et al., 2004). The concentrations of total polyphenolic compounds (TP) and total tannins (TT) in the samples were determined using the Folin–Ciocalteu assay in combination with polyvinyl–polypyrrolidone, using tannic acid (TA, Merck, Damstadt, Germany) as the reference standard (Makkar, 2003b). The condensed tannins (CT) content was determined by HCl-butanol oxidation (Porter et al., 1986). The TP and TT were expressed as *g* TA equivalent/kg DM, while CT was expressed as g leucocyanidin equivalent/kg DM. The HT content was estimated by subtracting CT from TT (Barman et al., 2008). To determine the concentration of HT derivatives in the feed samples (including GA, TA, EA, punicalin, and punicalagins A and B), 200 mg of PSP were solubilized in 10 mL of 80% methanol under constant shaking for 15 min (Gerhardt Co., model laboshake RO 300/08, Germany), and then centrifuged (Hettich Co., model EBA 20, Germany) for 10 min at 3000×*g*. The methanol was removed under N flow pressure and the aqueous residue was frozen and then used for HPLC analysis. A total 20 μL of the sample was injected onto HPLC (Yang Lin, model 100 system, South Korea) with a programmable wavelength photodiode array UV detector (280 nm) and C-18 column (5 μm, 25 μm × 0.46 μm Teknokroma). The solvents for elution were buffer A ($H_2O$, methanol, and $H_3PO_4$; 975.5:19.50:5.0 v/v/v) and buffer B ($H_2O$ and methanol; 300:700 v/v). The flow rate was maintained at 1.2 mL/min and the elution was monitored for collecting and analyzing three-dimensional chromatograms. The amount of each HT derivative was estimated by multiplying the percent of composition by the amount of TP (Gil et al., 2000).

### 2.2. Rumen DM degradability

Two Holstein heifers (400 ± 25 kg body weight (BW); mean ± SD), fitted with a flexible rumen fistula, fed a total mixed ration (TMR) twice daily (09:00 and 16:00 h), were used for incubation of the feed samples in rumen. The TMR included (on a DM basis) 2.7 kg of alfalfa hay, 1.75 kg of corn silage, and 2.25 kg of concentrate (containing 635.0, 58.0, 173.0, 100.0, 10.0, 4.0, 5.0, and 15.0 g/kg DM barley, cottonseed meal, beet pulp, wheat bran, limestone, salt, vitamin-mineral supplement, and urea, respectively) per heifer per day. To determine the DM degradability coefficients, 5 g of DM of each feed sample (ground using 2 mm screen mill) were placed in individual polyester bags (made of artificial silk cloth with a pore size of 50 μm and an average size of 12 cm × 19 cm) to give an approximate sample DM:surface area ratio of 11.5 mg DM:cm$^2$. The bags were placed in the dorsal sac of the rumen of the heifers immediately after the 09:00 h feeding (two bags for each feed were used, so eight bags at each incubation time were allocated per heifer). The feed samples were incubated for 2, 4, 6, 8, 12, 16, 24, 48, 72, and 96 h. After each incubation, the bags were hand-washed thoroughly in cold running water until the rinsing water was clear. Two bags of each feed were washed without incubation in the rumen (0 h samples). The bags were dried in a forced-air oven (58 °C, 48 h) and weighed to determine DM disappearance.

Visual examination of the disappearance curves for DM appeared to follow an exponential trend. Thus disappearance data for each feed sample were fitted to the model proposed by Ørskov and McDonald (1979) as follows: $p = a + b\,(1 - e^{-\mathrm{kd}^{*}t})$, where $p$ is the material lost from the bag at time $t$ (h), $a$ is the water soluble fraction (intercept with the *Y*-axis), $b$ is the potentially degradable (insoluble) fraction, and kd (1/h) is the fractional degradation rate of the $b$ fraction. The non-linear parameters were obtained by use of the PROC NLIN procedure of SAS Institute (2002).

The effective degradability (ED) of DM was estimated as follows: $\mathrm{ED} = a + (b \times \mathrm{kd})/(\mathrm{kd} + \mathrm{kp})$ where kp, which denotes the particulate outflow rate from the rumen, was set to 0.02, 0.05, and 0.08/h.