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Effect of ensiling pomegranate pulp with solid additives on chemical composition, intake and digestibility by sheep

D. Eliyahu[a,b], Y. Shaani[a,c], E. Yosef[a], Y. Ben-Meir[a], M. Nikbachat[a], R. Solomon[b], S.J. Mabjeesh[c], Z.G. Weinberg[d], J. Miron[a,*]

[a] Dept. of Ruminant Science, Agricultural Research Organization, P.O. Box 6, Bet Dagan 50250, Israel
[b] 'Ambar' Feed Mill, Granot, Israel
[c] Dept. of Animal Sciences, The Robert H. Smith Faculty of Agriculture, Food, and Environment, The Hebrew University of Jerusalem, Rehovot, Israel
[d] Forage Preservation and By-Products Research Unit, The Volcani Center, Agricultural Research Organization, Israel

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ABSTRACT

Effect of ensiling pomegranate pulp (PP) with or without solid additives on composition and *in vitro* digestibility was assessed in glass silos. Ensiling of fresh PP solely, resulted in low DM content (185 g/kg) and high fermentation losses (20% of DM). Notwithstanding, ensiling a mixture (on DM basis) of: 40% PP, 35% soy hulls and 25% corn silage, produced pomegranate pulp silage (PPS) characterized by low pH (3.86), appropriate DM content (34.7%), low fermentation losses during ensiling (5.58%) and similar *in vitro* DM digestibility (66.2%) compared with the raw material. Consequently, this PPS mixture was ensiled in two pressed bales (700 kg each) wrapped with stretch polyethylene film, for measuring PPS intake and digestibility by sheep. A preliminary observation demonstrated that lambs refused to consume diet based just on PPS and soybean plus minerals and vitamins mix. Only after reduction of PPS level in the TMR to 51% of the DM while replacing part of the PPS with wheat hay, the PPS-hay diet was entirely consumed. Therefore, four male Assaf lambs housed in individual metabolic cages, were fed ad-libitum two total mixed rations (TMR) in a cross over design: The PPS TMR contained 51% PPS, 38% chopped wheat hay, and 11% soybean plus minerals and vitamins mix; The reference Hay TMR contained 89% chopped wheat hay plus 11% minerals soybean plus minerals and vitamins mix. This hay-TMR was used to calculate the apparent digestibility of the PPS component in the PPS + Hay TMR. Lambs fed the PPS + hay TMR ingested voluntary 1,129 g DM/lamb/day including 580 g PPS DM/lamb/day. Apparent *in vivo* digestibility values of the PPS component in the experimental TMR were: dry matter, 60.1%; organic matter, 65.6%; crude protein, 47.1%; and neutral detergent fiber, 46.8%. Notwithstanding, *in vitro* DM and NDF digestibility of the PPS was higher (67.5% and 52.3%, respectively). The lower *in vivo* digestibility values compared with the corresponding *in vitro* data is associated with occurrence of soluble phenolics that are considered entirely digestible in the *in vitro* system but actually form non-digestible phenol–protein complexes in the sheep gut that reduce *in vivo* NDF and DM digestibility.

1. Introduction

Global production and consumption of pomegranate fruit have greatly increased in recent years up to 15 million metric tons (Fruit Market Reports, 2013), partly due to recognition of the health-promoting potential of various components of this fruit in human (Aviram et al., 2008). Pomegranate components have in animals wound-healing properties (Chidambara et al., 2004), immunomodulatory activity (Gracious et al., 2001; Shabtay et al., 2008), antibacterial activity (Navarro et al., 1996), as well as antiatherosclerotic and antioxidative capacities (Tzulker et al., 2007). Additional studies (Li et al., 2006; Tzulker et al., 2007) demonstrated higher antioxidant capacity of the peels relative to the juice, mainly due to water-soluble polyphenols, anthocyanins and hydrolyzable tannins (Gil et al., 2000; Tzulker et al., 2007). This led to development of advanced industrial technologies, which provide consumers with "ready to eat pomegranate grains and fresh

Abbreviations: ADF, acid detergent fiber; CP, crude protein; DM, dry matter; IVDMD, *in vitro* dry matter digestibility; IVNDFD, *in vitro* NDF digestibility; NDF, neutral detergent fiber; OM, organic matter; PP, fresh pomegranate pulp; PPS, silage of pomegranate pulp with solid additives; SEM, standard error of the means; TMR, total mixed ration; WSC, water-soluble carbohydrate.

* Corresponding author. Fax: +972 39604023.
E-mail address: jmiron@agri.gov.il (J. Miron).

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juices (Shabtay et al., 2012). This development increased global production of the byproduct fresh pomegranate pulp (PP), which is usually very wet (~20% DM) and may contain readily fermentable soluble sugars, resulting in spoilage under aerobic conditions. This spoilage may lead to unpleasant odors and attract flies, creating an environmental nuisance (Shabtay et al., 2008). Recently it was demonstrated that fresh PP was not stable under aerobic exposure and was readily contaminated by molds, yeast and other bacteria (Eliyahu et al., 2015). Disposal of this wet byproduct by drying or burying is not cost-efficient; a better alternative is to use it directly for feeding ruminants. However, processing of pomegranate fruit for PP production is limited in Israel to its short harvest season (September through December). Thus, seasonal limitations on the one hand, and high contents of moisture and fermentable sugars which interfere with preservation on the other, are the main obstacles for standardization of fresh PP as continuous and steady ingredient in ruminant rations.

In the present study, we developed an ensiling process that use solid additives to increase DM content of the fresh PP from 20% up to 36%, in order to improve its ensiling capability and reduce fermentation losses.

It was previously shown that supplementation of wet fresh PP (up to 200 g/kg dietary DM), promotes an increase in feed intake and a tendency to increase weight gain in bull calves (Shabtay et al., 2008). In addition, inclusion of up to 4% concentrated extract of pomegranate pulp in the TMR of lactating cows, increased their milk production and improved their health status (Shabtay et al., 2012). However, fresh pomegranate pulp contains high levels of soluble phenolics (27.3%) and NDF (41.3%) which impaired its voluntary consumption by sheep to 20% of a concentrated ration (Eliyahu et al., 2015). There is lack of information in the literature about the effect of ensiling fresh pomegranate pulp with or without solid additives on fermentation losses, chemical composition and digestibility of the silage in sheep.

This study aimed to: i. examine and compare changes in DM loss, *in vitro* digestibility and chemical composition during ensiling in glass silos of fresh pomegranate pulp solely or with solid additives; ii. examine voluntary intake and digestibility by sheep of pomegranate pulp silage (PPS) ensiled with soy hulls and corn silage as solid additives.

2. Materials and methods

2.1. Ensiling of pomegranate pulp

The fresh pomegranate pulp (PP) used in this study was obtained from the fruit juice factory, Primor, (Kibutz-Gat, Israel). A 2 kg portion of PP was frozen at −20°C until analysis (in four replicates) for composition and *in vitro* digestibility of the raw material (Table 1). In order to measure DM loss during ensiling, (ensiling is defined as anaerobic lactic fermentation that decreases pH) part of the fresh byproduct was ensiled in individual pre-weight 1 L glass-silos in four replicates (glass-silos) for each of the ensiling treatments: 1. either as the sole substrate without additives; 2. as a mixture (on DM basis) of 40% fresh PP, 35% soy hulls, and 25% corn silage. The glass silos were tightly closed with a glass cover and rubber ring, held with stretched metal holders that enabled the escape of pressed gases and volatiles while preventing the introduction of air into the vessel. The glass-silos were weighed, and stored for 60 days at room temperature (25 °C + 2). At the end of the ensiling period, the glass-silos were re-weighed and then opened for sampling. DM content was determined directly (in triplicate) on each replicate of the pre-ensiled raw materials and silage samples at 60° C for 48 h. Water extract was prepared manually (5 g DM/100 g water, in triplicates) from each replicate (four replicates) of the fresh pre-ensiled samples and silages to measure pH, lactate, volatile fatty acids, ethanol and water soluble carbohydrate (WSC) content as well as live count of yeast and moulds content. Pre-ensiled and silage samples (four replicates) were stored at −20 °C for further analyses of composition and *in vitro* digestibility (in triplicates). Recovery of DM in each glass silo was measured according to the quantitative balance between DM in the compacting source material and the outcome silage.

On March 10, 2013, 1.4 tons of PP with solid additives silage (PPS) were prepared at Massuot Yizkhak feeding center, with a baling machine (New Mix, Massuot Yizkhak, Israel). PP plus soy hulls plus corn silage (at DM ratio of 40:35:25, respectively) were premixed for 10 min in a mixing wagon (Lachish Industries Ltd., Sderot, Israel) and loaded into a compression chamber by a conveyor belt; the compressed bales were then lowered on to a rotating tray and wrapped with 8–9 layers of stretch polyethylene UV resistant film, 32 μm thickness (Poleg Industries, Kibbutz Gevim, Israel) as previously described (Weinberg et al., 2011). Two bales of about 700 kg each were prepared and stored outdoor at the feeding center until May 2013. Ambient temperatures were 25–28 °C during the outdoor storage period. The dimensions of the bales were 1.1 m × 0.9 m × 0.9 m. Composition of the PPS produced in bales was similar to that produced in the glass silos, and is presented in Table 1.

2.2. Analyses of chemical composition and development of yeasts and moulds

Dry matter content in each replicate of fresh PP, pre-ensiled raw materials, silages of each glass silo, dietary samples, orts, and dry feces samples from sheep, was determined (in triplicate) by drying in an air-forced oven for 48 h at 600 °C. Ash was determined (in triplicate) as the residue after 3 h at 600 °C (AOAC, 1980). WSC content in water extracts was determined (in triplicate) by the phenol–sulfuric acid method of Dubois et al. (1956). Lactic acid in the water extract was determined (in triplicate) by a spectrophotometric method according to Barker and Summerson (1941). Total water soluble phenolics in the water extract were determined (in triplicate) by the method of Kanner et al. (1994). Ethanol and volatile fatty acids in the water extract were determined by gas chromatograph equipped with a semi-capillary nitroterephthalic acid-modified polyethylene glycol (FFAP) column (Hewlett Packard, Waldborn, Germany) over a temperature range of 40–230 °C. pH was determined in each water extract of pre-ensiled materials and glass-silo silages with a pH-meter (PL 600, MRC, Netanya, Israel). Yeast and molds in each fresh water extracts were enumerated on spread-plate malt extract agar (Difco, Detroit, MI, USA) acidified to pH 4.0 with lactic acid; plates were incubated for 3 d at 30 °C, as described previously (Ashbell et al., 1991; Weinberg et al., 2011). Data of yeasts and moulds was presented as the logarithmus of colony forming units (CFU)/g DM substrate). Neutral detergent fiber (NDF) determined with heat-stable amylase and without Na-sulfite, acid detergent fiber (ADF) and acid detergent lignin were determined in dried (for 48 h at 60 °C), ground (1 mm sieve) samples according to the sequential method of Van Soest et al. (1991) by an ANKOM fiber analyzer ($ANKOM_{220}$ Technology, Macedon, NY, USA). Cellulose was calculated as ADF – ADL and hemicelluloses was calculated as NDF – ADF. Crude protein (CP) was determined according to the Kjeldahl method (procedure 14.068 in AOAC, 1980). Ether extract (EE) was determined according to AOAC procedure 14.131, (1980). These analyses were performed in triplicates and means are presented with standard error of the means (SEM).