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# Nutrient digestion, ruminal fermentation and performance of dairy cows fed pomegranate peel extract

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ABSTRACT

An experiment was carried out to determine the effect of pomegranate peel extract (PPE) on nutrient digestion, ruminal fermentation characteristics, protozoal population and performance of dairy cows. Four Holstein cows were used in a 4 × 4 Latin square design with 28-d periods and 4 treatments: PPE0 (no extract), PPE400 (400 ml PPE/cow/d), PPE800 (800 ml PPE/cow/d) and PPE1200 (1200 ml PPE/cow/d). Intake of dry matter, milk yield, and digestibility of dry matter, organic matter, crude protein, neutral detergent fiber and acid detergent fiber were measured. Ruminal fermentation characteristics such as ruminal pH, concentration of $NH_3$–N, concentration of VFA, molar proportions of individual VFA, protozoa population and microbial N were also measured. Milk production, 4% FCM yield, milk fat and protein yield (kg/d), and milk efficiency were increased by inclusion of PPE800 in the diet. Percent of milk fat, true protein, and lactose were not affected by PPE supplementation. However, inclusion of PPE decreased $NH_3$–N, total protozoal population, genus *Isotricha* and *Entodinium*, but increased microbial N production (g/d). Concentrations of total VFA and molar proportions of individual VFA were not affected by inclusion of PPE in the diet. The results suggested that PPE supplementation has reduced protozoa population, $NH_3$-Nconcentration, and increased microbial protein and milk yield and quality.



## 1. Introduction

Dry climatic condition and shortage of water resources in many countries, has led to a scarcity in the quantity and quality of consistent year-round supply of conventional ruminant feeds (Abarghuei et al., 2010). Consequently, the prices of animal feed, particularly protein supplements, become more costly. Yet, some common supplements, such as soybean meal, are metabolized less efficiently (i.e., losses of $NH_3$–N) in the rumen (Van Nevel and Demeyer, 1988) resulting in decreased animal performance and contributing to environmental pollution (Tamminga and Hobson, 1996). Thus, much research has been carried out to enhance the efficiency of protein metabolism and maximize the growth performance and economic viability of livestock operations. Plant secondary metabolites (PSM) in tree leaves such as *Salix babylonica*, *Leucaenaleucocephala*, and grape pomace extracts (Alipour and Rouzbehan, 2010; Jiménez-Peralta et al., 2011; Salem et al., 2011) were found to have a positive effect on ruminal fermentation parameters and to increase

*Abbreviations:* ADFom, ash-free acid detergent fiber; AIA, acid detergent insoluble ash; BW, body weight; CP, crude protein; CT, condensed tannin; DM, dry matter; HT, hydrolysable tannins; ME, metabolizable energy; N, nitrogen; $NE_L$, net energy for lactation; NDFom, ash-free neutral detergent fiber; NTP, non-tannin phenol; OM, organic matter; PD, purine derivates; PPE, pomegranate peel extract; PSM, Plant secondary metabolites; PVPP, polyvinylpyrrolidone; SP, saponin; TMR, total mix ration; TP, total phenols; TT, total tannin; VFA, volatile fatty acids

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**Table 1**
Ingredients and chemical composition (means ± SD) of the TMR fed to lactating cows ($n=4$).

| Ingredients (g/kg DM) | |
|---|---|
| Alfalfa hay | 229.0 |
| Corn silage | 211.9 |
| Barley, rolled | 134.5 |
| Corn grain, ground, dry | 81.9 |
| Wheat bran | 99.4 |
| Wheat grain, rolled | 27.7 |
| Soybean meal, 44% CP | 66.3 |
| Canola meal | 28.0 |
| Cottonseed meal | 39.3 |
| Vegetable oil | 12.4 |
| Limestone | 6.2 |
| Mineral+vitamin premix[a] | 12.4 |
| Salt | 6.2 |
| Molasses, beet sugar | 14.5 |
| Sodium bicarbonate | 12.4 |
| Fish meal | 17.0 |
| Chemical composition (g/kg of DM) | |
| DM | 603 ± 3.5 |
| OM | 926 ± 4.6 |
| CP | 160 ± 7.6 |
| NDF | 340 ± 2.5 |
| ADF | 201 ± 1.2 |
| $NE_L$ (Mcal/kg) | 1.57 ± 0.009 |

Estimated from NRC (2001).
[a] Contained 196 g Ca, 96 g P, 71 g Na, 19 g Mg, 3 g Fe, 0.3 g Cu, 2 g Mn, 3 g Zn, 100 ppm Co, 100 ppm I, 0.1 ppm Se and $50 \times 10^5$ IU vitamin A, $10 \times 10^5$ IU vitamin D and 0.1 g vitamin E/kg.

amino acid flow to the duodenum (Mueller-Harvey, 2006). This could lead to more muscle deposition and greater milk production (Vasta et al., 2008).

Pomegranate peel (PP) is a by-product of extracting the juice from pomegranates, with annual production of more than 120,000 t in Iran (Mirzaei-Aghsaghali et al., 2011). The PP contains secondary metabolites such as saponin, polyphenolic compounds, primarily punicalagin and ellagitannins, which have been shown to possess antimicrobial, antioxidant, anti-inflammatory, antimitotic, and immune modulatory properties (Adams et al., 2006; Oliveira et al., 2010). Bacterial predation by protozoa has the most deleterious effect on the efficiency of N use in the rumen. The PP contains saponins which may improve N efficiency by decreasing protozoal activity (Hess et al., 2004). Therefore, we hypothesized that inclusion of PP extract (PPE) to the diet would enhance ruminal microbial protein synthesis and increase milk protein content.

Oliveira et al. (2010) found that feeding a pomegranate extract to young calves for the first 70 d of life decreased intake of grains and whole tract digestibility of fat and crude protein, likely because of its high tannin content. However, Jami et al. (2012) and Shabtay et al. (2012) noted a significant increase in the digestibility of dry matter, crude protein, and neutral detergent fiber, as well as milk and energy-corrected milk yields in cows fed 4% pomegranate-peel extract supplement. The inconsistency between these studies may be ascribed to differences in pomegranate type (i.e., the concentration and nature of the active ingredients), extracting method the age of animals which affect animal performance. Commonly, extraction of the secondary metabolites is carried out using solvents, such as methanol, ethanol or acetone (Makkar, 2003), which is to some extent costly. Therefore, there is a need to investigate the effectiveness of less costly techniques at the farmer's level. Water can be used for extraction purposes because it is cheap and easy to handle. Hence, this experiment was carried out to assess the influence of three levels of PPE, extracted by water, on ruminal fermentation characteristics, protozoal population, microbial protein synthesis, nutrient digestibility and performance in dairy cows.

## 2. Materials and methods

### 2.1. Animal care

The experiment was carried out according to The Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) guidelines. All procedures and guidelines involving animals were approved by the Animal Experiment Committee at Tarbiat Modares University (Tehran, Iran).

### 2.2. Pomegranate peel extract

Pomegranate peel was obtained from two main factories in Saveh city, using similar pomegranate varieties and processing methods. Sun-dried peel was extracted at 1 g PP/ml of water. The peel was soaked in tap water at 40 °C for 72 h in a closed tank. To maximize the extraction of PSM (Table 2) from the PP, the tank was incubated in a water bath at 40 °C for one more hour. The contents, then, were immediately filtered and the filtrate was stored at 4 °C for further use.

### 2.3. Experimental design, cows and treatments

The experiment was designed as a balanced $4 \times 4$ Latin square for carryover effects, using 4 dairy cows with four 28-d periods. The cows in three lactations averaged 87 ± 29 DIM at the start of the experiment with a mean BW of 616 ± 53 kg. They were housed in individual tie stalls and had free access to water during the experiment. A TMR (Table 1) was fed for ad libitum intake (5–10% orts, on as-fed basis). The animals were randomly assigned to 1 of 4 treatments: (1) PPE0 (control, no PPE added), (2) PPE400 (400 ml PPE/cow per day), (3) PPE800 (800 ml PPE/cow per day), and (4) PPE1200 (1200 ml PPE/cow per day). The PPE was extracted daily. Each experimental period lasted 28 d with 2 d for adaptation to the diet, and 7 d for sampling and data collection. All diets were formulated to have similar concentrations of CP and $NE_L$ (NRC, 2001).

### 2.4. Feed intake, body weight and nutrient digestibility

Diets were offered in equal amounts 3 times daily (0600, 1400 h and, 2200 h). Feed consumption was recorded daily by weighing feeds offered to and refused by the cows. Samples of the TMR, feed ingredients, and orts were