

Nutritive and Antioxidative Potential of Fresh and Stored Pomegranate Industrial Byproduct as a Novel Beef Cattle Feed

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Pomegranate peel is a nutritive-rich byproduct whose amounts are extensively growing due to the exponential increase in the production of pomegranate juice and “ready to eat” arils. Pomegranate peel is a rich source for antioxidants and thus may serve in the prevention of cattle diseases and in the improvement of beef products, making it an attractive component in beef cattle diets. The present study aims to evaluate the effect of commonly used storage practices on the nutritive and antioxidative properties of pomegranate peel. In general, storage conditions preserved most antioxidant capacity. Ensiling ambivalently affected the nutritive values of the peel and promoted increased levels of antioxidative components. In addition to polyphenols, nonphenolic components, such as α - and γ -tocopherols, contributed to the total antioxidative capacity, and several minerals found in the peel added to its nutritional value. Dietary supplementation with fresh peels promoted significant increases in feed intake and α -tocopherol concentration in the plasma, with positive tendency toward increased weight gain of bull calves. All in all, the nutritive value and the antioxidant capacity of pomegranate peel turn it into a favorable health-promoting constituent of feedlot beef cattle diet.

KEYWORDS: Pomegranate peels; antioxidant activity; polyphenols; flavonoids; condensed tannins; hydrolyzable tannins; punicalagin; α -tocopherol; γ -tocopherol; minerals; storage conditions; average daily gain

1. INTRODUCTION

The global production and consumption of pomegranate has greatly expanded in recent years, together with the recognition of the health-promoting potential of various components of this fruit (1). These trends have led to the development of advanced industrial technologies that provide consumers with “ready to eat” pomegranate arils and with several fresh fruit juices. In turn, these advances have also led to the accumulation of a new

byproduct, namely, pomegranate peel. Pomegranate peel attracts attention due to its apparent wound-healing properties (2), immunomodulatory activity (3), antibacterial activity (4), and antiatherosclerotic and antioxidative capacities (5). Antioxidative activity has often been associated with a decreased risk of various diseases and mortality (6, 7). A positive correlation between oxidative stress and illnesses is widely documented in cattle (8, 9). Recent studies (5, 10) have demonstrated higher antioxidant capacity of the peel as compared with the aril juice. This antioxidant capacity has been mainly attributed to the water-soluble polyphenols, anthocyanins, and hydrolyzable tannins (5, 11). However, to our knowledge, lipid-soluble antioxidants, and especially vitamin E, have not been determined in pomegranate peel. Increased levels of vitamin E in diets for calves and cows have often been associated with improving their health status (12, 13) and the quality of their beef products (14, 15). Moreover, this property is even of greater significance when considering the recently reported finding of polyphenols pos-

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sessing synergistic properties with lipophilic antioxidants to prevent lipid peroxidation in the digestive tract (16, 17). In addition to the aforementioned compounds with antioxidant capacity, cells produce powerful antioxidant enzymes. The three major classes of antioxidant enzymes are superoxide dismutase (SOD), catalase, and glutathione (GSH) peroxidase (18), which require copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), and selenium (Se) for their catalytic activity. Thus, nutritive supplementation of these minerals may further promote resistance to oxidative stress.

The production of arils and concentrates for the juice industry is limited to the harvest season of pomegranate. To optimize the use of pomegranate peel in the beef and dairy industry, appropriate storage practices are sought. The aims of the present study were (i) to test the effect of different storage conditions on the nutritive potential and on various components of the antioxidant capacity of pomegranate peel and (ii) to test the effect of dietary supplementation with fresh peels on bull calves' weight gain and antioxidant parameters in their plasma.

2. MATERIALS AND METHODS

2.1. Pomegranate Peel—Source and Treatments. Freshly produced pomegranate peels of the 'Wonderful' cultivar were obtained from an industrial aril extraction facility (Mesilat Zion, Israel). The freshly obtained peels that contained up to 3% (w/w) of arils (peels + arils, P+A) served to test the effect of different storage conditions on the residual levels of antioxidant compounds as follows: A, 1 week at 4 °C refrigeration (Ref); B, 1 week in an open air shade (Air); C, oven drying for 48 h at 60 °C (Dry); and D, ensiling (Silage). Because of the washing process at the peeling facility, no fungal infestation took place. Pitted peels (Fresh) served to study the contribution of the left-over arils to the overall antioxidant capacity of peels. For anaerobic fermentation, fresh peels were chopped to 5 cm pieces and ensiled in 1.5 L Weck anaerobic jars (Wehr-Oflingen, Germany). The lids of the jars enabled gas release only but not air ingress. Each jar was filled with 850–900 g (wet weight) of peels without headspace, and the jars were stored at ambient temperature (22–25 °C) for 50 days. The air within the jars was replaced quickly by carbon dioxide due to the activity of aerobic microorganisms and by residual respiration of the biomass. This was a routine procedure in laboratory ensiling experiments. Dry matter (DM) losses caused by the anaerobic process were calculated by weight difference and the difference in DM content between day 0 and day 50.

In the case of P+A and only peels treatments, fresh samples were freeze-dried (Heto Drywinner, Heto-Holten, Denmark). Freeze drying was also applied as an end point to each treatment. All samples were freeze-dried and ground (Ika A11 Basic grinder; IKA Werke, Staufen, Germany; particle size, ~5 µm) prior to preservation, under vacuum, until analyzed. Comparison between treatments was carried out on DM basis. Biological replicates (3–5) from a randomized pool of 20 fruits each were taken for each treatment. Dry samples were stored in closed vials in the dark until analysis.

2.2. Chemical Analyses and Energy Concentration Calculation. Fresh and stored pomegranate peels were dried at 60 °C and assayed for DM, organic matter (OM), ether extract (EE), crude protein (CP), crude fiber (CF), ash (19), neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin (20). In vitro dry matter digestibility (IVDMD) (21) was performed to represent in vivo digestibility (22). NDF, ADF, and IVDMD were analyzed by using a ANKOM^R Fiber Analyzer (Fairport, New York) (23). Ash was determined in the samples before and after the IVDMD incubation to calculate the in vitro organic matter digestibility (IVOMD).

Metabolized energy (ME) was calculated according to NRC eqs 2-1 and 2-2 (24). Nitrogen free extract (NFE) was calculated by subtracting protein, EE, and CF concentrations from the OM concentration.

2.3. Antioxidant Activity Evaluation. We used the determination of TEAC (trolox equivalent antioxidant capacity) to evaluate the antioxidant capacity of pomegranate peel. TEAC was measured as 2,2'-azinobis(3-ethylbenzothiazolone-6-sulfonate) (ABTS^{•+}) decolorization, using preformed ABTS^{•+} radical cation, and calculated in accord to trolox as described previously (25). All determinations were performed in triplicate.

2.4. Colorimetric Determination of Total Phenolics. The amount of total polyphenols was determined in pomegranate peel using Folin–Ciocalteu reagent and calibrated against gallic acid (26). All tests were conducted in triplicate.

2.5. Colorimetric Determination of Flavonoids. Flavonoids were determined as described previously (27). Briefly, appropriate dilutions of sample extracts were reacted with NaNO₂ solution, followed by reaction with AlCl₃·6H₂O to form a flavonoid–aluminum complex. Subsequently, NaOH solution was added to the mixture. The absorbance was measured against prepared blank at 510 nm. The flavonoid contents were determined by a (+)-catechin standard curve and expressed as mean of mg (+)-catechin equivalents per 100 g of DM peel ± SD for at least three replications.

2.6. Colorimetric Determination of Condensed Tannin Content. Condensed tannins (procyanidins) were analyzed according to the method of Broadhurst and Jones (28). The amount of condensed tannin was expressed as milligrams catechin equivalents (mg of CAE/g sample) using the calibration curve of (+)-catechin.

2.7. Colorimetric Determination of Total Hydrolyzable Tannins. Plant material was extracted as described above, and gallic acid was determined before and after acid hydrolysis using the rhodanine assay (29).

2.8. Liquid Chromatography–Mass Spectrometry (LC-MS) Analysis of Punicalagin. Extraction and analysis of punicalagin from the peel were carried out as described elsewhere (5). Briefly, punicalagin was analyzed by a LC-MS instrument [Waters 2790 high-performance liquid chromatography (HPLC) system] equipped with a Micromass triple quadrupole Quatro-Ultima mass spectrometer. Chromatographic separations were carried out on a reverse phase ODS Hypersill column (2.1 mm × 100 mm, particle size 5 µm, Thermo catalog #30105-102130) using acetonitrile (A) and DDW (B) as the mobile phases, at a flow rate of 0.5 mL/min on a linear gradient mode. The solvent linear gradient started with 100% A, 3 min with 70% A, and 5 min with 100% A.

2.9. Analysis of Tocopherols. All preparations and analyses of tocopherols were carried out in the dark or under gold fluorescent light to avoid possible photodegradation of analyzed metabolites. All chemicals and solvents were purchased from Sigma (St. Louis, MO). Pomegranate peel samples were ground to a fine powder in the presence of liquid nitrogen, using a D3V-10 cutting mill (Hsiangtai Machinery Industry Co., Ltd., Taiwan) at 28000 rpm. A portion (0.5 g dry weight) of the powder was weighed for tocopherol analyses. Tocopherols were extracted from sample aliquots of about 0.5 g of finely ground pomegranate peel and fractionated with HPLC according to Tadmor et al. (30). Quantification of tocopherol was based on peak area obtained in a Waters 2475 Multichannel Fluorescence Detector. Quantification was performed by integrating the peak areas of the HPLC results using Millennium chromatography software (Waters, Milford, MA) assisted by standard curves of authentic standards (Sigma).

For the extraction of tocopherols from the plasma, 500 µL of plasma in 8 mL of hexane:acetone:ethanol (50:25:25) was vigorously shaken for 5 min, followed by the addition of 1 mL of NaCl in H₂O (25% w/v), vortexing, and shaking for 5 min. Subsequently, 8 mL of H₂O was added, and the samples were mixed by vortex and incubated for 10 min on the bench in dark. Finally, the upper phase was collected and re-extracted with an additional 2 mL of hexane, and the combined extract was evaporated to dryness in a Savant (Holbrook, NY) SpeedVac apparatus and resuspended in 400 µL of 45:5:50 (v/v/v) acetonitrile:methanol:dichloromethane. Samples were then filtered through a Syrasep (Intersep, Wokingham, United Kingdom) polytetrafluoroethylene syringe filter (0.2 µm) and kept at room temperature in the darkness for not more than 2 h before analysis by HPLC. Quantification of tocopherols in the plasma was carried out as mentioned above in this section.

2.10. Determination of Minerals. The concentrations of copper, iron, manganese, and zinc were determined by burning 1 g of dry treated

peels, solubilizing the ash in 70% HNO₃, diluting the preparation to 5% acid, and analyzing the preparation with an inductively coupled plasma-atomic emission spectrometer (Spectroflame; Spectro, Analytical Instruments, Kleve, Germany).

2.11. Animals and Feed Experimental Design. Twelve Holstein-Friesian bull calves participated in this study. The calves were divided randomly to two groups of six individuals, each housed in a different pen. The average body weights (BW) of the calves were 378 ± 46 and 385 ± 33 kg for the control and experiment calves, respectively. The average ages of the calves were 334 ± 21 and 321 ± 15 days for the control and experiment calves, respectively. The control group was served fattening ration ad lib (ME = 2.75 Mcal/kg DM, CP = 13.5%, and DM = 71%). The experiment group was served the same fattening ration, with the addition of ad lib pomegranate peels, offered as cafeteria. The peels were obtained once weekly from an industrial aril extraction facility (Mesilat Zion) and kept at 4 °C for the rest of the week. Feed was delivered each morning at approximately 7:00. Prior to the morning supplementation of new peel, the refused peel from the previous day was collected and weighted. Feed amounts and residuals were measured using a portable electronic scale (Carcom, Israel).

The experiment lasted 8 weeks. The intake of peels was measured once daily during the whole experiment period, whereas the intake of the fattening ration was measured in 2 week intervals. The fattening ration intake was adjusted to 24 h, to enable the calculation of average daily intake. At day 0 and in 2 week intervals, the calves were weighed.

2.12. Blood Sampling. In parallel to calf weighing, blood was sampled from the caudal vein, using evacuated tubes (Greiner bio-one GmbH, Austria) containing ethylenediaminetetraacetic acid as an anticoagulant. The blood was centrifuged at 1500g, 4 °C, to separate the cells from the plasma, which were immediately frozen in liquid nitrogen and kept at -70 °C until use.

2.13. Statistical Analysis. Differences in the values of all measured traits among treatments were tested by one-way analysis of variance (ANOVA) followed by Bonferroni's posthoc test ($P < 0.05$). The correlation between traits was carried out by the Pearson correlation coefficient. Paired *t* tests and correlation tests (Pearson) were performed to compare α - and γ -tocopherols in each treatment, both in peels and in plasma. Differences of average feed intake between control and peel-supplemented calves were tested by one sample *t* test. All variables were tested for their normality prior to analyses. The individual daily gain of each calf along the experiment period was calculated by slope function, and Student's *t* test was used to test the average daily gain (ADG) differences among control and peel-supplemented calves. All statistics were carried out by SPSS 15.0.

3. RESULTS AND DISCUSSION

3.1. Importance of Peel Preservation. The rapid increase in public awareness to the beneficial health traits attributed to pomegranate prompted a dramatic increase in its consumption and a demand for the introduction of juices and "ready to eat" arils. As a consequence, there is a growing production of pomegranate peels that are treated as waste but could be used as superior cattle feed. Indeed, pomegranate peel is recognized for its many health-promoting qualities (2–5). Individual active compounds in industrially produced pomegranate peels may constitute a desirable component in beef cattle diet, with emphasis on preventing morbidity and improving meat quality (12–15). However, fruits of the main commercial cultivar 'Wonderful' are available only during a relatively short season (October). Hence, this study aims to determine optimal storage conditions that would best preserve the advantageous peel ingredients for use in farm animal diets.

3.2. Polyphenol-Derived Antioxidative Capacity of Pomegranate Peel Stored under Various Conditions. Cold and air storage are usually used for short-term preservation, while oven drying and silage serve for long-term storage. Pomegranate peel is susceptible to spoilage because of high moisture and high

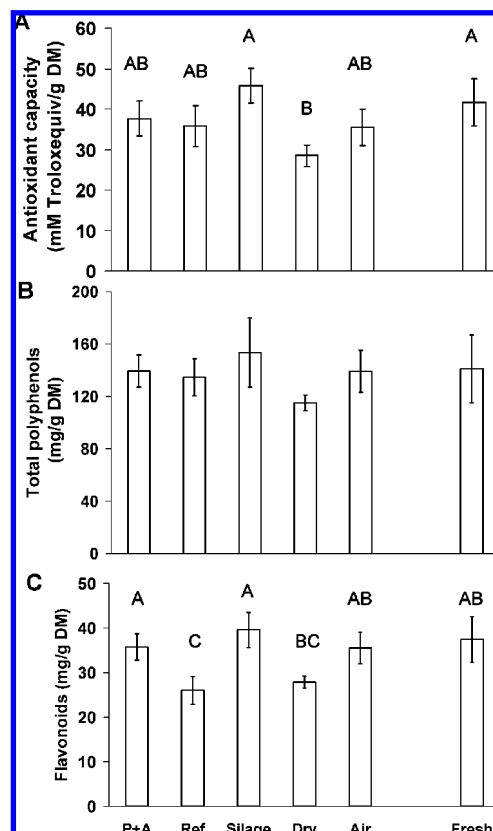


Figure 1. Effect of various storage conditions of pomegranate peels on their (A) antioxidant capacity, (B) total polyphenol content, and (C) total flavonoids content. P+A, fresh peels with aril left over; Fresh, peels only; ref, 1 week of storage at 4 °C; Silage, ensiled peels; Dry, oven-dried peels (60 °C, 48 h); and Air, 1 week of storage in the barn. Data are presented as means ± SDs of 3–5 replicates. Each of the replicates is a randomized pool of 20 fruits. Comparison was made on a DM basis. Letters indicate significant differences between treatments. Significant differences among treatments using Bonferroni's multiple comparison test ($P < 0.05$) are indicated by different letters above the bars (A > B).

carbohydrate and protein contents, making the short-term procedures less favorable. On the other hand, short-term storage may better preserve the antioxidant capacity of the peel. A well-performed long-term preservation may delay spoilage but could result in loss of beneficial traits.

To explore the effect of storage on the antioxidant capacity of peels, we used the ABTS free radical scavenging assay. Oven-drying treatment deleteriously affected the antioxidant capacity of the peels as supported by one-way ANOVA (Figure 1A, $F_{5,17} = 5.119$, $P < 0.005$). Fresh peels and peel silage showed a greater antioxidant capacity than oven drying, while no significant differences were found between other treatments.

It is commonly recognized that the antioxidant capacity in fruits is attributed to their content of polyphenols (5, 11, 31). Total polyphenols in peels, estimated by the Folin-Ciocalteu colorimetric assay, positively correlated with the antioxidant capacity, with $r = 0.9$, $n = 20$, and $P < 0.0005$ as obtained by the Pearson correlation test. Only oven drying resulted in reduced levels of polyphenols. Silage and short-term storage conditions did not affect polyphenol contents ($F_{5,18} = 1.417$, $p = 0.27$) (Figure 1B). A relative stability of total polyphenols in response to different drying methods (oven or freeze drying) was also demonstrated in artichoke (31). Notably, the arils did not contribute an additional value to the polyphenol content of the peels (Figure 1B).

The *in vitro* powerful antioxidant activities of flavonoids, such as scavenging a wide range of reactive oxygen, nitrogen, and chlorine species ions, and chelating metals to decrease their prooxidant activity have all been well-demonstrated. However, many studies show low concentrations of flavonoids in the plasma of mammals (ca. 1 $\mu\text{mol/L}$), leading to studies that argued for direct effects of flavonoids within the gastrointestinal tract (17). Alternatively, flavonoids may function not as hydrogen-donating antioxidants but rather through their binding to membranal and cellular receptors, influencing signal transduction and gene expression (32). Besides their antioxidant activity, flavonoids have long been recognized to possess anti-inflammatory and antiviral activities (32, 33) and thus could greatly reduce the incidence of certain cattle diseases.

The effect of storage conditions on the levels of flavonoids revealed significant differences between treatments as determined by one-way ANOVA ($F_{5,16} = 8.205$, $P < 0.001$). Refrigeration and dry treatments resulted in lower flavonoid contents, whereas air storage and ensiling did not affect flavonoid levels (Figure 1C). These findings indicate that flavonoids present in pomegranate peel exhibit differential sensitivity to various storage conditions. In agreement with the above results, the residual arils did not contribute to the total flavonoid levels (Figure 1C).

Pomegranate peel is also rich in tannins, yet another family of polyphenolics. Tannins are commonly classified into two classes, hydrolyzable and condensed tannins (also referred to as proanthocyanidins; 34). Tannins were shown to have both adverse and beneficial effects in ruminant animals (35). Moderate concentrations of condensed tannins (2–4% DM) in the diet improve production efficiency in ruminants, without increasing feed intake, as manifested by increases in wool growth, weight gain, milk yield, and ovulation rate (34). Here, refrigeration and oven drying had a deleterious effect on the content of condensed tannins in the peels, while silage doubled their concentration ($F_{5,13} = 34.867$, $P < 0.0001$; Figure 2A). Hydrolyzable tannins are considered the dominant component of the antioxidant activity of pomegranate juice (11). Recently, hydrolyzable tannins were shown to correlate positively with antioxidant activity and polyphenols content in pomegranate peel (5). Oven-drying treatment deleteriously affected the content of hydrolyzable tannins in the peels, while silage doubled their concentration ($F_{5,16} = 22.814$, $P < 0.0001$) (Figure 2B). As effluent losses after 50 days of ensiling were $15.2 \pm 1.1\%$ (w/w) and DM losses were rather high ($37 \pm 5\%$), the doubled value revealed for tannins during the anaerobic process might be the result of a concentration effect in the silage. In pomegranate, the hydrolyzable tannins include punicalin, ellagic acid, gallagic acid, and punicalagin, which account for 50% of the antioxidant capacity of pomegranate juice (11). Of the hydrolyzable tannins in pomegranate peel, punicalagin has the strongest lipid peroxidation inhibitory and radical scavenging activities (36), and its health benefit attributes may be of great relevance to cattle health (37). We have compared the effect of storage conditions on the content of punicalagin in peels. As shown in Figure 2C, all storage conditions significantly decreased the content of punicalagin in the peels ($F_{5,14} = 15.263$, $P < 0.0005$). These results indicate that, unlike total polyphenols, short- or long-term storage conditions of pomegranate peels lead to reduced punicalagin content. Indeed, no correlation was found between punicalagin and total polyphenols upon conservation of the peels ($r = 0.23$, $n = 20$, and $P = 0.329$). However, despite its significant decrease in conserved peels, punicalagin still constitutes a large portion of the polyphenols in peels (Figure 2C).

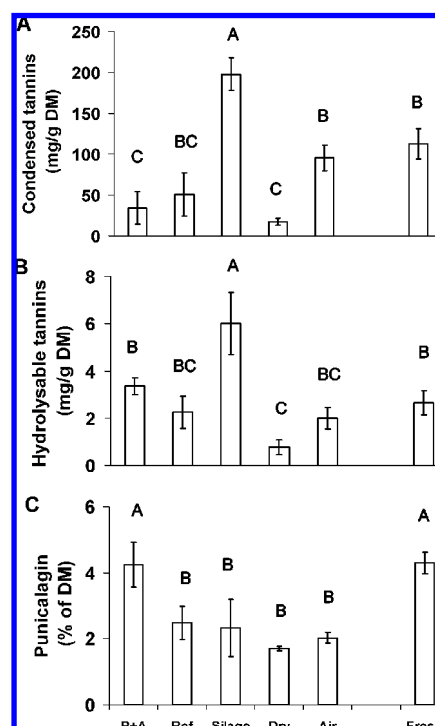


Figure 2. Effect of various storage conditions of pomegranate peels on their (A) total condensed tannins content, (B) total hydrolyzable tannins content, and (C) punicalagin content. Data are presented as means \pm SDs of 3–5 replicates. Each of the replicates is a randomized pool of 20 fruits. P+A, fresh peels with aril left over; Fresh, peels only; ref, 1 week of storage at 4 $^{\circ}\text{C}$; Silage, ensiled peels; Dry, oven-dried peels (60 $^{\circ}\text{C}$, 48 h); and Air, 1 week of storage in the barn. Letters indicate significant differences between treatments. Significant differences among treatments using Bonferroni's multiple comparison test ($P < 0.05$) are indicated by different letters above the bars ($A > B > C$).

In general, storage of pomegranate peel affects similarly the various polyphenolic components and their antioxidant capacities. Silage was found to preserve the highest levels, while oven-drying treatment significantly decreased the levels of the antioxidative components of pomegranate peels.

3.3. Nonpolyphenolic Antioxidative Components in Pomegranate Peels. Assessment of the antioxidant activity and the total polyphenol content of pomegranate juice and of whole pomegranate homogenates showed that polyphenols could not account for ca. 3-fold of the increase in antioxidant activity of the whole fruit extract (5). Decker et al. (38) suggested that an accurate evaluation of the antioxidative potential of food products should take into account factors such as physical location of antioxidants. To evaluate the contribution of non-polyphenolic phytochemicals, we determined the level of vitamin E in the peel. Vitamin E comprises multiple stereoisomers of α -, β -, γ -, and δ -tocopherols and four tocotrienols (39), with tocopherols α and γ being its major components. In pomegranate peel, both α -tocopherol and γ -tocopherol are present (Figure 3). A great portion of γ -tocopherol is contributed by the arils. Indeed, in peels that contained no arils, the levels of γ -tocopherol were significantly lower than in any of the other treatments ($F_{5,15} = 52.593$, $P < 0.0005$). γ -Tocopherol levels were relatively stable under the various storage conditions, unlike the polyphenolics, which were susceptible to the oven-drying treatment. Moreover, under silage conditions, the γ -tocopherol concentration has significantly increased. In comparison to all aril-containing treatments ($t_3 = -3.85$, $P = 0.031$; $t_3 = -5.444$, $P = 0.012$; $t_2 = -7.311$, $P = 0.018$; $t_1 = -6.651$,

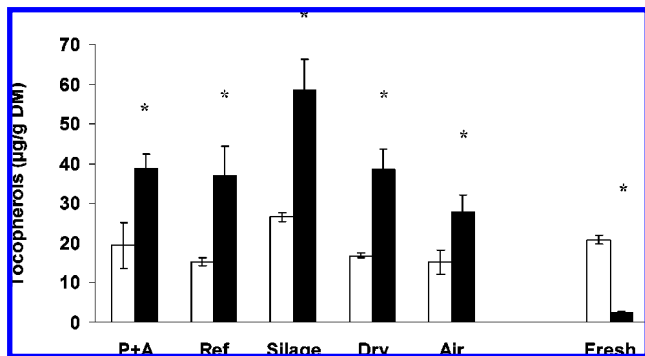


Figure 3. Effect of various storage conditions of pomegranate peels on their α -tocopherol (open bars) and γ -tocopherol (black bars) contents. Data are presented as means \pm SDs of 3–5 replicates. Each of the replicates is a randomized pool of 20 fruits. P+A, fresh peels with aril left over; Fresh, peels only; ref, 1 week of storage at 4 °C; Silage, ensiled peels; Dry, oven-dried peels (60 °C, 48 h); and Air, 1 week of storage in the barn. Asterisks indicate significant differences ($P < 0.05$, using a t test) between α - and γ -tocopherol within the same treatment.

$P = 0.095$; and $t_2 = -7.468$, $P = 0.017$ for P+A, refrigeration, silage, oven drying, and air treatments, respectively), the levels of α -tocopherol in the nonaril containing peels were higher than the levels of γ -tocopherol ($t = 36.937$, $P < 0.0005$). A recent study (40) demonstrated 25-fold higher levels of α -tocopherol as compared with γ -tocopherol in fruit peel from plants of the Mediterranean region. α -Tocopherol did not change under all storage conditions. However, significant differences in α -tocopherol contents were measured when comparing silage to air and refrigeration treatments ($F_{5,17} = 6.052$, $P < 0.002$).

3.4. Mineral Content of Stored Pomegranate Peel. Copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and selenium (Se) are all involved in the enzymatic neutralization of free radicals. Supplementing the diet with these trace elements was shown to elevate their blood levels as well as the activities of antioxidative enzymes in mammals (41). For example, calves that were fed milk supplemented with 25 ppm Cu and 100 ppm Zn showed a stronger immune response and a higher SOD activity (41). Here, the levels of Cu, Fe, Mn, Zn, and Se were determined in fresh and stored pomegranate peel. As expected, the changes in mineral levels among the different treatments were minor and varied as follows: Cu, 2.4–7.5 $\mu\text{g/g DM}$; Fe, 4.1–22.6 $\mu\text{g/g DM}$; Mn, 2.9–22.9 $\mu\text{g/g DM}$; and Zn, 4.9–13.6 $\mu\text{g/g DM}$. Selenium was not detected. As the fresh treatment possessed a significantly lower concentration of Fe, in comparison to the others, it is noteworthy that a significant fraction of Fe originated from the arils. We believe that the above-described results fit well with the high nutritive value of pomegranate peels for cattle feeding and its relative stability toward all of the treatments applied here. Altogether, these results indicate that pomegranate peel, fresh or stored, may serve as a good source for specific minerals (42).

3.5. Nutritive Value of Fresh and Ensiled Pomegranate Peel. In cattle feedlot facilities, fodder is supplemented to the animals either fresh or in their preserved form as silage. On that basis, and as most antioxidative ingredients were best recovered in the silage, our initial aim was to compare the nutritive value of fresh and ensiled pomegranate peel. Feedstuffs are evaluated on the basis of their ability to elicit the requisite product response from the domestic animal. The availability of nutrients in a feed is essentially determined by its chemical composition, with respect to available and unavailable components and to factors that may limit the availability of components with which they are associated. By measuring and calculating

Table 1. Chemical Composition, OM Digestibility, and Calculated ME of Fresh and Ensiled Pomegranate Peels^a

	fresh peels	ensiled peels	P^b
DM (%)	18.7 \pm 0.5	14.5 \pm 0.3	<0.001
ash (%)	6.3 \pm 0.3	7.5 \pm 0.4	<0.05
protein (%)	5.8 \pm 0.3	9.2 \pm 0.7	<0.01
NDF (%)	19.3 \pm 0.3	29.8 \pm 0.8	<0.001
ADF (%)	15.9 \pm 0.5	26.5 \pm 1.3	<0.001
EE (%)	2.3 \pm 0.4	2.1 \pm 0.4	0.5
lignin (%)	4.5 \pm 0.2	8.9 \pm 0.9	<0.01
CF (%)	14.2 \pm 0.2	23.2 \pm 0.8	<0.01
NFE (%)	71.3 \pm 0.3	58.1 \pm 0.3	<0.001
IVOMD (%)	76.2 \pm 0.4	63.0 \pm 0.7	<0.001
ME (Mcal/kg DM)	2.8	2.2	<0.05

^a Data are presented as means \pm SDs of four replicates. Values appear as % of DM. ^b t test for two independent samples.

Table 2. Composition of the Fattening Mix Rations (g/kg of DM)

component	concentration
barley grain	122
crushed maize grain	244
vetch hay	206
wheat silage	112
concentrate premix ^a	300
minerals and vitamins ^b	16
ME concentration (Mcal/kg)	2.75
CP (g/kg)	145

^a Wheat, 45.3%; wheat bran, 33%; and gluten feed, 21.7%. ^b Composition per 1 kg of minerals and vitamins premix: calcium carbonate, 450 g; salt, 350 g; dicalcium phosphate, 150 g; Mn, 1.1 g; Zn, 1.1 g; Fe, 1.1 g; Cu, 0.45 g; I, 0.3 g; Co, 7.5 mg; Se, 11 mg; vitamin A, 375000 IU; vitamin D₃, 75000 IU; and vitamin E, 200 IU.

protein, NDF, ADF, NFE, and EE contents and by evaluating ME and IVOMD, the nutritive value of feed can be estimated for its incorporation into the diet.

The DM, chemical composition, OM digestibility, and metabolizable energy of fresh and ensiled pomegranate peels are shown in **Table 1**. As compared to fresh peels, ensiled peels gained on protein, NDF, ADF, lignin, and CF but lost DM, OM digestibility, and ME. The ensiling process of the pomegranate peel was characterized by massive effluent losses. This, together with the fermentative costs, may well affect the content of NFE, leading to a decreased concentration of metabolizable carbohydrates in the silage. Indeed, loss of NFE indicates that carbohydrates are being fermented (43). As a result, DM, digestibility, and ME significantly decreased. The ME values of fresh and ensiled peel were 2.94 and 2.35 Mcal/kg DM, respectively (**Table 1**).

Pomegranate peel is of high energetic value as compared to selected feedstuffs commonly fed to cattle (NRC 2001), such as alfalfa (ME = 1.96 Mcal/kg DM), almond hulls (ME = 1.89 Mcal/kg DM), apple pomace (ME = 1.86 Mcal/kg DM), barely grain (ME = 2.92 Mcal/kg DM), barely silage (ME = 2.03 Mcal/kg DM), citrus pulp (ME = 2.76 Mcal/kg DM), corn silage (ME = 2.33 Mcal/kg DM), cotton seed (ME = 2.91 Mcal/kg DM), sorghum silage (ME = 1.79 Mcal/kg DM), and tomato pomace (ME = 2.37 Mcal/kg DM). The ME and OM digestibility values of fresh peel, and to a lesser extent of ensiled peel, may justify their utilization in mixed ration of bull calves in considerable amounts.

3.6. Feeding Beef Calves with Pomegranate Peels. To test the actual nutritive and antioxidative values of fresh pomegranate peel, we conducted a feeding experiment in which fresh peels (P+A) were offered to bull calves ad lib as cafeteria. Fresh pomegranate peel, in the present study, contained 140 mg total

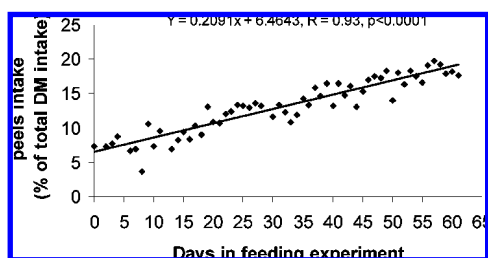


Figure 4. Effect of linear increase of consumed pomegranate peels by Holstein-Friesian bull calves, on a DM basis, as a function of experiment duration.

polyphenols /g material on a DM basis, of which hydrolyzable and condensed tannins constituted 3.35 and 34.4 mg/g, respectively, on a DM basis (**Figures 1B** and **2A,B**). Tannins are considered to have both adverse and beneficial effects in ruminant animals (35). High concentrations of tannins may reduce intake, digestibility of protein and carbohydrates, and animal performance through their negative effect on palatability and digestion (44). By preventing bloat and increasing the flow of nonammonia nitrogen and essential amino acids from the rumen (45), low and moderate (20–45 mg/g DM) concentrations of condensed tannins in the diet improved production efficiency in ruminants, without increasing feed intake. This was manifested by increases in wool growth, weight gain, milk yield, and ovulation rate (34). To test the motivation of bull calves to consume the tannin-rich pomegranate peel, we followed its voluntary intake for 2 months. Pomegranate peels intake of calves in the current study increased linearly during the feeding experiment and was ca. 20% of the total feed intake, on a DM basis, after 60 days (**Figure 4**). At this time point, the actual calculated intake of peel-derived tannins was 6.88 and 0.67 mg/g condensed and hydrolyzable tannins, respectively, on a DM basis. The linear increase over time in pomegranate peel consumption (**Figure 4**) demonstrates their palatability to beef calves. Principally, in spite of their palatability, the peels could exert negative effects on calves' feed intake and digestion, as often referred to tannin-rich feeds. It is shown in **Figure 5A** that these amounts of peel-derived tannins did not exert adverse effects on the average daily feed intake. On the contrary, peel-consuming calves had significantly higher feed intake during the entire experiment period ($p < 0.001$) (**Figure 5A**). The difference between average daily feed intake of calves consuming either control or peel amended diets originated from the net peel consumption of the experimental calves, on top of their fattening ration intake. This higher feed intake is further reflected in the higher ADG of the peel-consuming calves (**Figure 5B**). Thus, pomegranate peel intake of up to 20% of the total feed intake does not possess deleterious or positive effects on fattening ration intake of feedlot calves. However, because of its palatability, in these amounts, total feed intake is increasing, and its nutritional value consequently adds to the ADG. In spite of the significantly higher feed intake of the peel-consuming calves, their ADG only tended toward significance. As feed intake was not individually measured (but grouped for six individuals), this tendency to significance in ADG might be explained by the variability of peel intake among calves in the peel-consuming group. Indeed, our observations indicate differential feeding behavior of the peels, dictated by the dominant calves in the group.

Feeding diets that are supplemented with vitamin E reduced lipid peroxidation in beef (15), diminished the incidence of pulmonary diseases in feedlots (12), and had the potential to prevent undesirable reactions during beef storage (14). Previ-

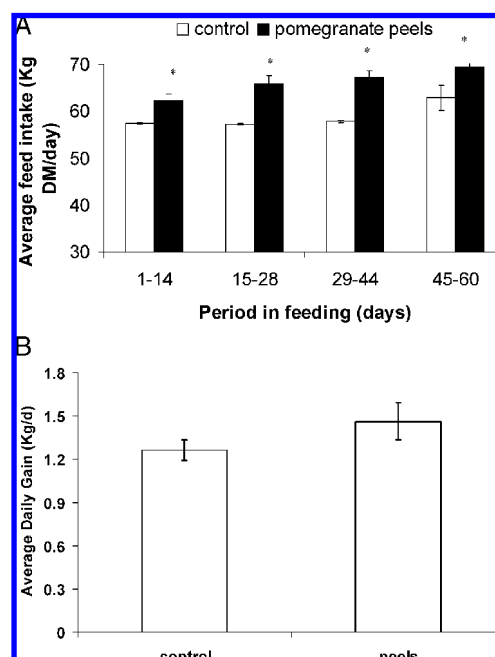


Figure 5. Effect of pomegranate peel supplementation in the diet of Holstein-Friesian bull calves on their average daily feed intake (**A**) and ADG (**B**). Results are presented as means \pm SDs. Asterisks indicate significant differences ($P < 0.001$) using *t* tests.

Table 3. Plasma α -Tocopherol Concentration in Control and Peel-Consuming Calves along the Feeding Experiment^a

weeks in feeding experiment	concentration of α -tocopherol (μ g/mL)	
	control	peels
0	1.75 \pm 0.19	1.72 \pm 0.29 a
2	1.69 \pm 0.11	2.25 \pm 0.32 ab
4	1.76 \pm 0.11	2.71 \pm 0.41 bc
8	1.82 \pm 0.15	2.92 \pm 0.36 c

^a Data are given as means \pm SDs. Different letters indicate significant differences within the peel-consuming group.

ously, it was shown that dietary supplementation of 4000 mg/day vitamin E for 90 days resulted in ca. 3-fold increase of α -tocopherol in muscle membranes of Holstein-Friesian bull calves (14). In the present study, the concentration of α -tocopherol in the fresh peel, on a DM basis, was ca. 20 μ g/g (**Figure 3**). The maximal calculated average daily intake of peel derived α -tocopherol, on a DM basis, was 46.7 mg and occurred in the last 2 weeks of the feeding experiment. In spite of the relatively low peel-derived α -tocopherol intake, a Pearson correlation test revealed a significant correlation between duration of the feeding experiment and increasing concentration of α -tocopherol in the plasma ($R = 0.86$, $n = 5$, and $p = 0.03$). No correlation was found in the control calves ($R = -0.05$, $n = 5$, and $p = 0.47$). Furthermore, a paired *t* test revealed that the levels of α -tocopherol in the plasma of peel-consuming calves already tended toward a significant increase after 2 weeks ($t_4 = -2.102$, $p = 0.052$) and turned significant after 4 weeks ($t_4 = -3.186$, $p = 0.017$) of consumption. Again, no changes in α -tocopherol levels have occurred in the plasma of control calves ($t_4 = 1.055$, $p = 0.176$ and $t_4 = 1.305$, $p = 0.131$ for 2 and 4 weeks of feeding, respectively). The corresponding concentrations of plasma α -tocopherol are given in **Table 3**. Prior to introducing peels to the experimental group, and as also judged by the control group, levels of α -tocopherol were slightly above minimal recommended concentration (1.5 μ g/mL) for cattle (46). At the end of the feeding experiment, this value was almost doubled.

Increased levels of α -tocopherol in plasma and other tissues, over time, in response to its supplementation in the diet, have been widely reported (14, 47, 48). However, relative to these studies, the increase in plasma α -tocopherol in the current manuscript is not derived from elevating its amount in the ration but might have been exerted by other mechanisms.

Two complementary mechanisms may explain the rapid accumulation of α -tocopherol in the plasma, in spite of relatively low levels and short period of peel-originated α -tocopherol intake: (i) As demonstrated in the current study, as well as in others (5, 11), pomegranate peel exhibits a broad spectrum of polyphenolic antioxidative activity. Recently (16), it was demonstrated that polyphenols protect various antioxidants, including β -carotene, ascorbic acid, and α -tocopherol, from oxidation in a physiological digestive environment. It is, thus, reasonable to assume that the protective property of polyphenols over antioxidants, from oxidation in the digestive tract, may have increased the availability of α -tocopherol in the plasma. Accordingly, the peel-derived polyphenols may have positively affected the efficacy of α -tocopherol absorption from the diet. (ii) Freshly obtained peels (P+A) contain a small amount of arils. As seen in **Figure 3**, a significant amount of γ -tocopherol is contributed by the aril. All forms of vitamin E are absorbed similarly in the intestine and enter the circulation packed into chylomicrons (49). Supplemental γ -tocopherol, as in the case of arils, may thus buffer some degradation of α -tocopherol in the digestive tract, leaving more of it available in the plasma after passage through the liver.

In summary, the data presented here indicate the high value of the pomegranate byproduct as cattle feed. The current study provides evidence for the potential of preserving the nutritive and antioxidative traits of pomegranate peel as beef cattle feed. Pomegranate peels contain a broad spectrum of desirable ingredients, previously shown to be relevant in the prevention of cattle diseases and in the improvement of beef products. It is demonstrated herein that arils do not contribute a significant portion of these ingredients. Although affecting the levels of the various components, the storage conditions tested in this study did not lessen much of the overall antioxidant capacity of pomegranate peel. Among the storage conditions, silage has promoted increased levels of most of the parameters tested, presumably due to the marked increase in its polyphenol-associated CFs content. At least in the case of fresh peel, it is well-demonstrated in the present study that their consumption has positive effects both on calves' growth and on plasma accumulation of α -tocopherol. Simply because a nutrient is directly involved with the antioxidant system does not necessarily mean that its supplementation in the diet improves cattle health. However, the bioavailability of minerals, vitamins, and polyphenols in fresh and stored pomegranate peels, if used in balance, may elicit in cattle beneficial prophyllactic effects.

ABBREVIATIONS USED

SOD, superoxide dismutase; GSH, glutathione; DM, dry matter; OM, organic matter; EE, ether extract; CP, crude protein; CF, crude fiber; NDF, neutral detergent fiber; ADF, acid detergent fiber; IVDMD, in vitro dry matter digestibility; ME, metabolized energy; NFE, nitrogen free extract; TEAC, trolox equivalent antioxidant capacity; ADG, average daily gain.

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