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POTENTIAL NUTRITIVE VALUE AND ANTI-METHANOGENIC POTENTIAL OF POMEGRANATE PEEL FOR SHEEP

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Abstract: The aim of the current experiment was to determine potential nutritive value and anti-methanogenic potential of pomegranate peels obtained from 12 different pomegranate fruits in Turkiye. There are significant variations among pomegranate peel (PP) samples in terms of chemical composition, gas production, methane production, metabolisable energy (ME) and organic matter digestibility (OMD). The crude ash (CA), ether extract (EE), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and condensed tannin (CT) contents of PP samples ranged from 2.99 to 4.72%, 2.11 to 5.25 %, 2.53 and 6.36%, 20.79 to 27.29%, 11.71 and 17.96%, 0.79 to 3.39% respectively. Gas production and CH4 production of PP samples ranged from 41.07 to 57.22, 5.29 and 7.87 respectively whereas percentage of CH4 ranged from 12.51 and 14.03%. The ME and OMD of PP samples ranged from 7.92 to 10.84 MJ/kg and 54.43 to 68.95% respectively. Although PP samples studied in the current experiment have the low CH4 reduction potentials, the CP contents of PP samples are not sufficient to meet minimum level of CP requirement for sheep. Therefore protein supplementation is required for optimum rumen function and feed intake in ruminant animals when large amount of PP samples were included into ruminant diets. However before large implication, there is a need for *in vivo* experiment to test the mitigating effect of PP samples.

Keywords: Pomegranate peel, Chemical composition, Digestibility, Methane production

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1. Introduction

Pomegranate (*Punica gratum* L) is one of important edible fruit in most of parts of world (Seeram et al., 2006). After processing of pomegranate in juice industry, considerable amount of a by-product such as pulp or peel become available. Recently pomegranates by products have attracted great attention due to phenolic compounds (Jami et al., 2012). Although there are some researches about the use of pomegranate by products such as pulps or peels in ruminant diets to meet their nutrient requirements during the conventional feed shortage, otherwise being a wasted product (Shabtay et al., 2008, Canbolat et al., 2014; Kotsampasi et al., 2014, Omer et al., 2019; Moradi et al., 2020; Elmorsy et al., 2022) there is limited information about the nutritive value of pomegranate peel from different sources. Johnson and Johnson (1995) suggested that considerable amount of dietary energy lost occurs through enteric fermentation which is one of great contributors to greenhouse gasses. Recently some investigations showed that CT in feedstuffs had a mitigating effect on methane production (Bodas et al., 2012; Bhatta et al., 2013; Hixson et al., 2016). The pomegranate byproducts contain CT which may possible affect the enteric methane production when included into ruminant diets (Moradi et al., 2020). The chemical composition in company with in vitro gas production have been used to evaluate potential nutritive value and anti-methanogenic potential of previously uninvestigated feedstuffs (Kamalak and Canbolat, 2010; Jayanegara et al., 2011; Kaplan, 2011; Uslu, 2018). The aim of the current experiment was to determine potential nutritive value and anti-methanogenic potential of pomegranate peel obtained from 12 different pomegranate fruits in Turkiye.

2. Material and Methods

2.1. Pomegranate Peel Samples

In the current experiment pomegranate peel samples obtained from 12 different pomegranate fruits through removing the seed by hand from different parts of Turkiye and dried in shade until the constant weight (Table 1). The pomegranate peel samples were ground to pass 1 mm sieve for the subsequent analysis.

2.2. Chemical Analysis of Pomegranate Peel Samples

Pomegranate peel samples were analyzed separately in for DM, CA, EE contents of GP (AOAC, 1990). NDF and ADF contents of PP samples were analyzed with the method suggested by Van Soest (1991). The CT contents of PP samples were analysed with buthanol-HCl method (Makkar et al., 1995).

Table 1. Pomegranate peel samples obtained from 12 different pomegranate fruits

PPS	Site
PP1	Kahramanmaraş, Türkiye
PP2	Kahramanmaraş, Türkiye
PP3	Van, Türkiye
PP4	Kahramanmaraş, Türkiye
PP5	Kahramanmaraş, Türkiye
PP6	Kahramanmaraş, Türkiye
PP7	Kahramanmaraş, Türkiye
PP8	Şırnak, Türkiye
PP9	Şırnak, Türkiye
PP10	Şırnak, Türkiye
PP11	Konya, Türkiye
PP12	Antalya Türkiye

PPS= pomegranate peel samples.

2.3. Determination of Gas and Methane Production of Pomegranate Peel Samples

Approximately 200 mg PP samples were weighted into glass syringes in triplicate and subjected to fermentation with 40 ml of the buffered rumen fluid (1:2 V/V) in the bath set at 39 °C for 24 h incubation using in vitro gas production technique (Menke et al., 1979) to determine gas and methane production. The rumen fluid was obtained from slaughter house and filtered with four layered cheesecloth under flushing with CO_2 before use. The methane content (%) of gas was determined using infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goel et al., 2008). The methane production of pomegranate peel samples as mL was calculated as follows (equation 1);

$$CH_4 \text{ production (ml)} = \text{Total gas production (ml)} \times \text{percentage of } CH_4 (\%) \quad (1)$$

The ME and OMD of PP samples were calculated using the equations indicated by Menke and Steingass (1988) (equations 2 and 3);

$$ME \text{ (MJ/kg DM)} = 0.72 + 0.15597GP + 0.068CP + 0.249EE \quad (2)$$

$$OMD (\%) = 15.38 + 0.8453GP + 0.595CP + 0.675CA \quad (3)$$

GP: gas production of 200 mg sample at 24 h incubation (ml), CP: crude protein (%), EE: ether extract (%), CA: crude ash (%)

2.4. Statistical Analyses

Data obtained current study was subjected to one-way analysis of variance (ANOVA) to determine the effect of source on chemical composition, in vitro gas production, methane production, ME, OMD of PP samples. Differences ($P<0.05$) among the mean were determined with Tukey tests (Genc and Soysal, 2018).

3. Results and Discussion

The chemical compositions of PP samples are given in Table 2. There are significant variations among PP samples in terms of chemical composition. The crude ash contents of PP samples ranged from 2.99 in PP7 to 4.72% in PP3. The crude ash contents of PP samples are consistent of findings of Moradi et al. (2020) and Omer et al. (2019).

Ether extract contents of PP samples ranged from 2.11 to 5.25 % with highest being in PP12.

The crude ash contents of PP samples are in agreement with those reported by Moradi et al. (2020) and Omer et al. (2019).

Crude protein contents of PP samples ranged from 2.53 and 6.36 % with highest being in PP12 and lowest in PP2. Except for PP12, CP contents of PP samples are lower than those reported by Moradi et al. (2020) and Omer et al. (2019) but consistent with that by Mirzaei-Aghsaghali et al. (2011).

Van Soest (1994) suggested that crude protein contents of diets should be higher than the minimum level of 7-8% of DM for optimum rumen function and feed intake in ruminant animals. Therefore, the CP content of PP samples is not sufficient to meet minimum level of CP requirement.

Table 2. The chemical composition of pomegranate peel samples obtained from sources

Type	DM	CA	EE	CP	NDF	ADF	CT
PP1	89.90^{ab}	4.33^{b}	2.11^{c}	3.41^{cde}	23.52^{bcde}	15.25^{cd}	1.82^{bcd}
PP2	90.79^{a}	4.19^{b}	2.53^{c}	2.53^{e}	25.17^{a}	18.38^{a}	1.69^{bcd}
PP3	86.90^{cd}	4.72^{a}	2.90^{c}	4.54^{bc}	23.90^{bcd}	13.65^{f}	1.21^{cd}
PP4	88.67^{abc}	3.55^{de}	3.43^{abc}	3.84bcd	22.52^{cdefg}	11.71^{g}	3.39^{a}
PP5	88.80^{abc}	3.01^{f}	2.83^{c}	3.84^{bcd}	21.73^{efg}	11.92^{g}	2.50^{abc}
PP6	89.58^{ab}	3.40^{de}	3.56^{abc}	3.80^{bcd}	21.75^{defg}	13.65^{f}	1.68^{bcd}
PP7	85.82^{d}	2.99^{f}	4.81^{ab}	4.64^{b}	20.75^{g}	14.03^{ef}	1.05^{d}
PP8	90.04^{ab}	4.32^{b}	3.76^{abc}	3.45^{cde}	22.81^{cdefg}	15.03^{cde}	2.98^{ab}
PP9	87.91^{bcd}	3.99^{bc}	2.73^{c}	3.81^{bcd}	20.97^{fg}	14.30^{def}	2.54^{abc}
PP10	86.25^{d}	3.75^{cd}	3.16^{bc}	3.32^{de}	23.04^{bcdef}	15.46^{c}	0.79^{d}
PP11	88.86^{abc}	3.55^{de}	2.31^{c}	2.81^{de}	24.39^{bc}	16.84^{b}	1.98^{bcd}
PP12	85.95^{d}	3.38^{e}	5.25^{a}	6.36^{a}	27.29^{a}	17.96^{a}	1.73^{bcd}
SEM	0.600	0.100	0.508	0.328	0.601	0.305	0.395
Sig.	***	***	***	***	***	***	***

[a,b]Column means with common superscripts do not differ at $P>0.05$. SEM= standard error mean.

Therefore protein supplementation is required for optimum rumen function and feed intake in ruminant animals when large amount of PP samples were included into ruminant diets.

The NDF contents of PP samples ranged from 20.79 to 27.29 with highest being in PP12 and lowest being in PP8 samples. The ADF contents of PP samples ranged from 11.71 and 17.96% with highest being in PP4 and 5 and lowest being in PP2 and 12 samples. NDF and ADF contents of PP samples were lower than those reported by Moradi et al. (2020) and Omer et al. (2019).

Condensed tannin contents of PP samples varied between 0.79 and 3.39 % with highest being in PP4 and lowest in PP10 samples. The effects of CT in diets depend on the amount and chemical structure (Makkar, 2003, Min et al., 2003, Mueller-Harvey, 2006, Goel et al., 2005, Tavendale et al., 2005; Galindo et al., 2008; McSweeney et al., 2011, Min et al., 2014). Low level of CT in diets may exert beneficial effect by preventing of protein from extensive degradation in the rumen but high CT level (6 and 10% of DM) reduces intake and growth of animal (Barry et al 1984). It is likely that all PP samples studied in the current experiment may have beneficial effect since their CT content lower than detrimental level. As can be seen from Table 2 there is a large variation among PP samples in terms of chemical composition. These variations among PP samples are likely related to type of pomegranate and growing site.

Gas production, CH_4 production, ME and OMD of PP samples are given in Table 3. There are significant variation among PP samples in terms of Gas production, CH_4 production, ME and OMD. Gas production and CH_4 production of PP samples ranged from 41.07 in PP2 to 57.22 in PP4, 5.29 in PP2 and 7.87 in PP4 respectively whereas percentage of CH_4 ranged from 12.51 in PP7 and 14.03% in PP5. The ME and OMD of PP samples ranged from 7.92 in PP2 to 10.84 MJ/kg in PP4 and 54.43 in PP2 to 68.95 % in PP4 ml respectively. Gas production and ME values of PP samples obtained in the current experiment were considerably higher than those of PP silage samples reported by Hatami et al. (2015) whereas OMD values was lower than those of PP silage samples reported by Hatami et al. (2015). On the other hand gas production and ME values of PP samples obtained in the current experiment was consistent with that reported by Mirzaei-Aghsaghali et al. (2011).

Lopez et al. (2010) the percentage of CH_4 of gas produced after 24 h incubation can be used to determine the CH_4 mitigation potential of feedstuffs. As can be seen from Table 3, PP samples studied in the current experiment have the low CH_4 reduction potential since the % of CH_4 in gas fell into range between 11 to 14% which is low potential group suggested by Lopez et al. (2010).

Table 3. The gas production, methane production, metabolisable energy and organic matter digestibility of pomegranate peel samples obtained from sources

Type	Gas	CH_4(ml)	CH_4 (%)	ME(MJ/kg/DM)	OMD (%)
PP1	47.36^{d}	6.55^{d}	13.85^{ab}	8.86^{bcd}	60.37^{bcde}
PP2	41.07^{d}	5.29^{a}	12.93^{ab}	7.92^{d}	54.43^{e}
PP3	50.10^{bcd}	6.73^{abcd}	13.44^{ab}	9.56^{abc}	63.62^{abcd}
PP4	57.22^{a}	7.87^{a}	13.78^{ab}	10.84^{a}	68.95^{a}
PP5	52.84^{ab}	7.41^{ab}	14.03^{a}	9.92^{abc}	64.36^{abc}
PP6	42.71^{cd}	5.95^{cd}	13.90^{ab}	8.52^{cd}	56.04^{de}
PP7	50.38^{abcd}	6.31^{bcd}	12.51^{b}	10.08^{ab}	62.75^{abcd}
PP8	51.74^{abc}	6.86^{abc}	13.27^{ab}	9.96^{abc}	64.09^{abcd}
PP9	52.84^{ab}	6.89^{abc}	13.01^{ab}	9.89^{abc}	65.01^{abc}
PP10	50.65^{abcd}	6.67^{abcd}	13.13^{ab}	9.63^{abc}	62.67^{abcde}
PP11	44.63^{bcd}	5.61^{cd}	12.60^{ab}	8.44^{cd}	57.17^{cde}
PP12	52.02^{abc}	6.72^{abcd}	12.92^{ab}	10.57^{a}	65.74^{ab}
SEM	2,800	0.415	0.420	0.436	2.371
Sig.	***	***	***	***	***

^{a,b}Column means with common superscripts do not differ at P>0.05. SEM= standard error mean.

4. Conclusion

There is considerable amount variation among PP samples in terms of chemical compositions, in vitro gas production, CH_4 production, ME and OMD. PP samples studied in the current experiment have the low CH_4 reduction potential whereas the CP content of PP samples is not sufficient to meet minimum level of CP requirement. Therefore protein supplementation is required for optimum rumen function and feed intake in ruminant animals when large amount of PP samples were included into ruminant diets. However before large implication, there is a need for *in vivo* experiment to test the mitigating effect of PP samples.

Author Contributions

Concept: İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%), Design: İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%), Supervision: İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%), Data collection and/or processing: İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%), Data analysis and/or interpretation:

İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%), Literature search: İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%), Writing: İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%), Critical review: İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%). Submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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