Influence of the addition of different levels of tannin extracted from pomegranate pomace, on some nutritive value of soybean meal

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ABSTRACT

This study was carried out to determine effect of addition of different levels of tannin extracted from pomegranate pomace, on some nutritive value of soybean meal (SBM) through gas production test. Sheep fitted with fistula were fed with experimental feed containing SBM treated with extracted tannin at levels of 1.5, 3, 4.5 and 6 g per kg dry matter. Cumulative gas production was recorded at 2, 4, 6, 8, 12, 16, 24, 36 and 48 h of incubation. The organic matter digestibility (OMD), short chain fatty acid (SCFA), metabolizable energy (ME) and net energy for lactation (NE\textsubscript{L}) were calculated from gas production after 24h incubation. The gas volume was significantly (P<0.01) highest for untreated SBM followed respectively by SBM treated with 1.5%, 3% of extracted tannin from pomegranate pomace. Significantly (P<0.01) lowest gas production was recorded from SBM treated with 4.5% and 6% of tannin extract. The gas production of soluble and insoluble fractions (a+b) of SBM was significantly (P<0.01) reduced when SBM was treated with 4.5% and 6% of tannin extract. The prediction of OMD, SCFA, ME and NE\textsubscript{L} were significantly (P<0.01) reduced when SBM treated by 4.5% and 6% extracted tannin. The current study concluded that the tannin treated SBM decreased the gas production and decreased the gas production from (a+b) of SBM.

Key words: Pomegranate pomace, gas production, tannin, soybean meal.

INTRODUCTION

Soybean meal (SBM) is the most commonly used protein supplement in livestock feeds. It is very palatable and has a good amino acid balance with high availability. Its bypass essential amino acid index is just next to ruminal microbial protein surpassing all other undegradable protein sources [1]. Pomegranate (Punica granatumL.) is one of the oldest edible fruits and has been used extensively in the folk medicine. Popularity of pomegranate has increased tremendously especially in the last decade because of its anti-microbial, anti-viral, anti-cancer, potent anti-oxidant, and anti mutagenic. These potential properties of the fruit are due to presence of different antioxidants such as ascorbic acid, carotenoids, flavonoids, and hydrolysable tannins [2] . However, pomegranates also have some anti-nutritional factors such as tannins and other secondary compounds. Tannins have high affinity for proteins and protect them from ruminal microbial degradation [3,4]. Tannins are polyphenolic substances with various molecular weight and a variable complexity. Negative effects of tannin on ruminant nutrition have been studied for several years in terms of palatability, feed intake, decreased digestibility, decreased production, decreased nutrient utilization, particularly protein [5]; damage of kidney and liver [6]; tissue damage in the rumen, intestine ulceration and morphological changes at the microvilli level [7]. However, besides these anti-nutritional and toxic effects, there is an increasingly awareness of tannin’s beneficial roles on animal nutrition and health, namely influences on the cell signaling pathways, anti-oxidative effects and anti-helmintic and anti-microbial activities. In ruminants important positive effect of tannins is dietary protein protection from ruminal microflora attack [8]. In vivo, in situ and in vitro methods have been used to evaluate the nutritive value of feedstuffs. The in vitro gas production technique has proven to be a potentially useful technique to evaluate the nutritive value of feedstuffs, since it gives
an estimate of the potential rate and extent of nutrient fermentation in the rumen. However, this technique is measuring gas produced by the fermentation of energy containing components in feeds, and not only that of protein [9,10,11]. The objective of the present study was to determine the effect of several levels of extracted tannin (1.5%, 3%, 4.5% and 6%) from pomegranate pomace on estimated some parameters of soybean meal by using gas production test.

MATERIALS AND METHODS

Reagents and chemicals
Folin-Ciocalteu phenol reagent (Sigma Chemical Co), Sodium carbonate, ethanol, tannic acid was obtained from Merck.

Sample collection
Pomegranate pomace (PP) samples were collected from Spoota factories in Urmia West Azerbaijan province, Iran. Different parts of the fruits, rind and seeds were extracted with aqueous ethanol (80% v/v) using a pomace-to-solvent ratio of 1:4 (w/v).

Tannin extract preparation
A fresh sample of PP (500 g DM/kg fresh weight) was dried and ground to pass a 1 mm sieve. Fifty grams (oven dry matter, Gallenkamp, UK) of PP was treated with 120 ml of an aqueous solution of ethanol (80%). The mixture was heated for 5 minutes. After heating, the pomace was cooled, washed and filtered through filter paper. Then 100 ml of the ethanol was again added to pomace and heated for 10 minutes and filtered through filter paper. The washed liquid was evaporated to a moderate concentration (rotary evaporator, temperature: 50 °C) in 30 minutes.

Determination of total phenolics (TP) content of pomegranate pomace (PP)
Aliquots (1 ml) of samples of the extracts were mixed with 5 ml Folin-Ciocalteu reagent in 100 ml volumetric flasks that contained 70 ml of deionised water. Sodium carbonate solution (15 ml of 20% m/v anhydrous sodium carbonate in deionised water) was added after 1 min within 10 minutes. The volumetric flasks were then made up to volume with deionised water. After standing for 2 h at room temperature, the absorbance was read at a wavelength of 640 nm in the visible range of the spectrum using a UV/Visible spectrophotometer (Ultrospec 2100 pro, Biochrom Ltd., Cambridge, England). The estimation of total phenols in the extracts was carried out in triplicate. Tannic acid was used as a standard on various concentrations and the results obtained were expressed as mg tannic acid equivalent/g of sample, on a dry weight basis. The TP content was determined according to the Folin–Ciocalteu method reported by [12].

Animals and feeding
Two rumen-fistulated Ghezel sheep (live weight 61±1.5 kg) were used. The sheep were fed a diet containing of 60% hay and 40% concentrate during one month before the experiment.

Treatments
The treatments were soybean meal (SBM), SBM treated with 15 g of tannin extract/kg dry matter (DM), SBM treated with 30 g of tannin extract/kg DM, SBM treated with 45 g/kg DM of tannin extract and SBM treated with 60 g/kg DM of tannin extract.

Chemical Analyses
Protein source were analyzed according to (AOAC., 1990) for DM, crude protein (CP), crude fat (CF) and ash in (table 1). The phytochemicals from various parts of the pomegranate tree and from pomegranate fruits and seeds are listed in (Table 2). The physical and chemical properties of pomegranate have been evaluated by [14,15]. On an average basis the pomegranate has following components as shown in (Table 3):

<table>
<thead>
<tr>
<th>Table 1. The chemical composition of soybean meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>feed</td>
</tr>
<tr>
<td>SBM</td>
</tr>
</tbody>
</table>

1: Dry matter, 2: Crude protein, 3: Ether extract, 4: Ash
Table 2. Phytochemicals of Pomegranate

<table>
<thead>
<tr>
<th>Part</th>
<th>Phytochemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pomegranate Juice</td>
<td>anthocyanins, glucose, ascorbic acid, ellagic acid, gallic acid, caffeine acid, catechin, EGCG, quercetin, rutin; numerous minerals, particularly iron; aminoacids.</td>
</tr>
<tr>
<td>Pomegranate seed</td>
<td>oil 95-percent punicic acid; other constituents, including ellagiacid; other fatty acids; sterols.</td>
</tr>
<tr>
<td>Pomegranate pericarp (Peel, rind)</td>
<td>Phenolic punicalagins; gallic acid and other fatty acids; catechin, EGCG; quercetin, rutin and other flavonoids; flavones, flavonones; anthocyanidins.</td>
</tr>
<tr>
<td>Pomegranate leaves</td>
<td>Tannins (punicalin and punicaloin); and flavones glycosides, including luteolin and apigenin</td>
</tr>
<tr>
<td>Pomegranate flower</td>
<td>Gallic acid, ursolic acid; triterpenoids, including maslinic and Asiatic acid; other unidentified constituents</td>
</tr>
<tr>
<td>Pomegranate roots and bark</td>
<td>Ellagitannins, including punicalin and punicalagen; numerous piperidine alkaloids.</td>
</tr>
</tbody>
</table>

Table 3. Pomegranate Chemical Composition

<table>
<thead>
<tr>
<th>Food Value</th>
<th>Percentage</th>
<th>Minerals &amp; Vitamins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture 78.0%</td>
<td></td>
<td>Calcium</td>
</tr>
<tr>
<td>10 mg Protein 1.6%</td>
<td></td>
<td>Phosphorus</td>
</tr>
<tr>
<td>70 mg Fat 0.1%</td>
<td></td>
<td>Iron</td>
</tr>
<tr>
<td>0.3 mg Minerals 0.7%</td>
<td></td>
<td>Vitamin C</td>
</tr>
<tr>
<td>16.0 mg Fibre 5.1%</td>
<td></td>
<td>Vitamin B complex</td>
</tr>
<tr>
<td>Trace amounts Carbohydrates 14.5%</td>
<td></td>
<td>--</td>
</tr>
</tbody>
</table>

In vitro gas production and rumen fermentation

Ruminal fluid was obtained from two fistulated sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). Equal volumes of ruminal fluid from each sheep were collected 2 h after the morning feeding and squeezed through four layers and mixed with McDougall buffer prewarmed to 39° C [16]. The inoculums was dispensed (20 ml) per vial into 50 ml serum vial (containing of 300 mg sample per vial) which had been warmed to 39° C and flushed with oxygen free CO2. The vials were sealed immediately after loading and were affixed to a rotary shaker platform (lab-line instruments Inc, Iran) set at (120 rpm) housed in an incubator. Vials for each time point, as well as blanks (containing no substrate), were prepared in triplicate. Gas production was measured in each vial after 2, 4, 6, 8, 12, 16, 24, 36 and 48 h of incubation using a water displacement apparatus [17]. Cumulative gas production data were fitted to the model [18,19]:

\[ P = a + b(1 - e^{-ct}) \]

Where: \( P \) is the gas production from the immediately soluble fraction (ml), a: the gas production from the insoluble fraction (ml), b: the gas production rate constant for the insoluble fraction (b), t: the incubation time (h) and P: the gas production at the time t.

The metabolizable energy (MJ/kg DM) content of soybean meal was calculated using equations [19,20,21] as follows:

\[ \text{ME} (\text{MJ/kg DM}) = 1.06 + 0.157 \text{GP} + 0.084 \text{CP} + 0.22 \text{CF} - 0.081 \text{CA} \]

Where:

\( \text{GP} \) = The 24 h net gas production (ml/200 mg DM)

\( \text{CP} \) = Crude protein

Short chain fatty acids (SCFA) are calculated using the equation [22,23].

\[ \text{SCFA (mmol)} = 0.0222 \times \text{GP} - 0.00425 \]

The organic matter digestibility (OMD) was calculated using equations [21] as follows:

\[ \text{OMD} (\% \text{ DM}) = 14.88 + 0.889 \text{GP} + 0.45 \text{CP} + 0.0651 \text{XA} \]
Where:

\( GP = \text{About 24 h net gas production (ml /200 mg DM)} \)

\( CP = \text{Crude protein (\%)} \)

\( XA = \text{Ash content (\%)} \)

The net energy for lactation (\( NE_L \)) was calculated using equations [4] as follows:

\[
NE_L (\text{Mcal/lb}) = \frac{(2.2 + (0.0272 \times GP) + (0.057 \times CP) + (0.149 \times CF))}{14.64}
\]

Where: \( GP \) is 24 h net gas production (ml/g DM)

\( CP \) is crude protein (% of DM)

\( CF \) is crude fat (% of DM)

Then the NE unit was converted to MJ/Kg DM.

Statistical Analysis

Data were subjected to one way analysis using a general linear model (GLM) procedure of SAS vision (9.1), with Duncan's multiple range test used for the comparison of means in gas production test and The least significance different (LSD) was used to compare the data and all tests were considered significantly different at \( p \leq 0.05 \) in Determination of total phenolics (TP) content.

RESULTS AND DISCUSSION

Chemical Composition

The chemical composition of SBM is presented in (Table 1).

Total phenolics content

Standard curve was obtained from plotting of light absorption against different concentration of tannic acid and are shown in (Figure 1) and the equation of light absorption is shown in (table 4). The contents of total phenolics of pomegranate pomace extract are given in (Table 5).

![Figure 1. Standard curve was obtained from plotting of light absorption against different concentration of tannic acid](image)

**Table 4. Equation of light absorption against different concentration of tannic acid**

<table>
<thead>
<tr>
<th>Standard curve</th>
<th>equation</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannic acid standard</td>
<td>( Y = 0.0004x - 0.0023 )</td>
<td>0.977</td>
</tr>
</tbody>
</table>

\( x \) concentration (in milligrams/milliliters), \( Y \) absorbance at 640 nm
Reference [24] reported that in pomegranate peel extracts the amount of phenolic compounds in the acetone extracts in either solvent or ultrasound-assisted solvent extraction methods were the highest (40.0 and 35.0 % for sonication and solvent extraction, respectively (P< 0.05)), followed by methanol (34.5 and 31.0 %), ethanol (25.3 and 23.0 %), water (10.0 and 12.0 %), and ethyl acetate extracts (0.2 and 0.2 %). Ethyl acetate extraction gave the highest content of the TP (20.24%), proanthocyanidins (2.65%) and flavonoids (3.92%) in the extracts. Reference [25] reported that the highest content of the TP (20.24%), proanthocyanidins (2.65%) and flavonoids (3.92%) in the extract. The phenolic composition of pomegranate depends on multiple actors, including climate and variety. Pomegranate pomace extract was rich in total phenolic content and showed its potential for antioxidant activity and could be further evaluated as dietary supplements.

The gas production study

The cumulative gas production “corrected for blank fermentation” of soybean meal (SBM), SBM treated with 1.5% tannin extract, SBM treated with 3% tannin extract, SBM treated with 4.5% tannin extract, and SBM treated with 6% tannin extract that were incubated in buffered rumen fluid from sheep fed the ration with alfalfa hay and concentrate is showed in (Figure 2). The mean values of cumulative gas production during 48 h of SBM treated with tannin extract is presented in (Table 6). It has been reported that tannins decrease cumulative gas production [26]. Similar results were obtained [27], who found that adding tannic acid to SBM decreased gas production.

![Figure 2. In vitro gas production volume of SBM and SBM treated whit tannin extracts (15, 30, 45, 60 g/kg DM) in different incubation times](image)

**Table 6. In vitro gas production (ml/g DM)**

<table>
<thead>
<tr>
<th>Incubation times (h)</th>
<th>parameters of GP (a+b)</th>
<th>( C^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>45.31(^a)</td>
<td>0.092</td>
</tr>
<tr>
<td>4</td>
<td>43.22(^b)</td>
<td>0.088</td>
</tr>
<tr>
<td>6</td>
<td>39.51(^c)</td>
<td>0.090</td>
</tr>
<tr>
<td>8</td>
<td>35.11(^d)</td>
<td>0.094</td>
</tr>
<tr>
<td>12</td>
<td>31.22(^e)</td>
<td>0.095</td>
</tr>
<tr>
<td>14</td>
<td>1.62</td>
<td>4.09</td>
</tr>
</tbody>
</table>

1: soybean meal, 2: SBM treated with 15 g/kg dry matter (DM) of tannin extract, 3: SBM treated with 30 g/kg DM of tannin extract, 4: SBM treated with 45 g/kg DM of tannin extract, 5: SBM treated with 60 g/kg DM of tannin extract, 6: Standard error of the mean, 7: a potential gas production (ml/g DM), 8: constant rate of gas production (ml/h). a,b,c Within a column, means without a common superscript letter differ (P < 0.01).

The significantly (P<0.01) highest mean values of cumulative gas production during 48 h was obtained by SBM followed by SBM treated with 1.5% tannin extract, SBM treated with 3% tannin extract. The significantly (P<0.01) lowest gas production obtained by the treatment whit 4.5% and 6% tannin extract.
The estimated kinetics parameters by exponential model are presented in (Table 6). The soluble and insoluble fractions (a+b) of SBM were significantly (P<0.01) decreased when SBM was treated by 4.5% and 6% tannin extract. The mean values (a+b) were 301.66, 300.47, 296.96, 278.57 and 264.65 ml respectively for SBM, SBM treated with 1.5%, 3%, 4.5% and 6% tannin extract. The mean value (a+b) of SBM treated with 4.5% and 6% tannin extract was significantly (P<0.01) lower compared with untreated SBM.

The predicted ME (MJ/kg DM), NE\textsubscript{L} (MJ/kg DM), OMD (%), SCFA (mmol) from gas production are presented in (Table 7). The predicted ME and NE\textsubscript{L} which calculated from gas production after 24 h incubation were decreased when SBM was treated by 1.5%, 3%, 4.5%, 6%, tannin extract. The mean values of ME were 13.4, 13.41, 13.36, 12.94 and 12.43 MJ/kg DM for untreated SBM, SBM treated with 1.5%, 3%, 4.5% and 6% tannin extract respectively.

\begin{table}[h]
\centering
\caption{Estimated parameters}
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{feed} & \textbf{ME\textsuperscript{a}} & \textbf{NE\textsubscript{L}\textsuperscript{b}} & \textbf{OMD\textsuperscript{c}} & \textbf{SCFA\textsuperscript{d}} \\
\hline
SBM & 13.44 & 7.92 & 83.63 & 1.14 \\
SBM 1.5\% & 13.41 & 7.90 & 83.45 & 1.13 \\
SBM 3\% & 13.36 & 7.87 & 83.17 & 1.13 \\
SBM 4.5\% & 12.94 & 7.64 & 80.82 & 1.07 \\
SBM 6\% & 12.43 & 7.36 & 77.92 & 0.99 \\
SEM & 0.107 & 0.058 & 0.608 & 0.015 \\
\hline
\end{tabular}
\end{table}

1: soybean meal, 2: SBM treated with 15 g/kg dry matter (DM) of tannin extract, 3: SBM treated with 30 g/kg DM of tannin extract, 4: SBM treated with 45 g/kg DM of tannin extract, 5: SBM treated with 60 g/kg DM of tannin extract, 6: Standard error of the mean, 7: metabolizable energy ME MJ/kg DM; 8: net energy for lactation (MJ/kg DM), 9: organic matter digestibility (%), 10: short chain fatty acid (mmol).

a,b,c Within a column, means without a common superscript letter differ (P < 0.01).

The mean values of NE\textsubscript{L} were 7.92, 7.90, 7.87, 7.64 and 7.36 MJ/kg DM for untreated SBM, SBM treated with 1.5%, 3%, 4.5% and 6% tannin extract respectively. NE\textsubscript{L} were significantly (P<0.01) reduced when SBM treated by 4.5% and 6% tannin extract respectively. The mean values of OMD were 83.63, 83.45, 83.17, 80.82 and 77.92 % for untreated SBM, SBM treated with 1.5%, 3%, 4.5% and 6% tannin extract respectively and the mean values of SCFA were 1.14, 1.13, 1.13, 1.07 and 0.99 mmol for untreated SBM, SBM treated with 1.5%, 3%, 4.5%, and 6% tannin extract respectively. These results are in agreement with the earlier researchers [4,27] when SBM treated with tannin. Reference [28] also reported that OMD, SCFA and ME are reduced when processing of sunflower meal with tannic acid. The decrease in OMD, ME and NE\textsubscript{L} was probably due to increased Maillard reaction that decreased rumen degradability and formation of complexes between tannins and dietary proteins and carbohydrates, as well as reducing rumen microbial proteolytic, ureolytic and cellulolytic enzyme activities, general fermentative activities and cell multiplication [29]. Reference [30] reported that tannins significantly decreased gas production probably hampering rumen microorganisms.

Acknowledgement

The authors gratefully thank the Islamic Azad University Tabriz Branch and Ms. Arezoo pourali for her assistance.

REFERENCES