*Full Length Research Paper*

# Estimation of the nutritive value of tomato pomace for ruminant using *in vitro* gas production technique

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An abundance of agricultural by-products have been used to feed dairy and beef cattle in Iran, in order to reduce the cost of livestock production. The aim of this study was to determine the chemical composition and estimation of nutritive value of dried tomato pomace (DTP) using *in vitro* gas production technique. Fermentation of DTP samples were carried out with rumen fluid obtained from three mature canulated steers. The samples were collected from "San San Shahd" factory in Urmia, Iran. The amount of gas production for DTP at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h were measured. The results showed that the crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and non fibrous carbohydrate (NFC) contents were 26.4, 50.6, 34.5 and 3.7%, respectively. Gas volume at 24 h incubation (for 200 mg dry samples), soluble fraction (a), insoluble but fermentable fraction (b), potential gas production (a + b) and rate constant of gas production (c) contents were 39.38, -1.05, 52.96, 51.90 and 0.081 ml h$^{-1}$, respectively. The organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) and net energy for lactation (NE$_l$) contents were 62%, 9.06 MJ kg$^{-1}$ DM, 0.0.869 mmol and 4.51 MJ kg$^{-1}$ DM, respectively. According to results of this study, it seems that DTP could be used as a valuable food industrial by-product in ruminant nutrition.

**Key words:** Nutritive value, gas production, tomato pomace, short chain fatty acid, metabolizable energy.

## INTRODUCTION

Feeding by-products of the crop and food processing industries to livestock is a practice as old as the domestication of animals by humans. It has two important advantages, these being to diminish dependence of livestock on grains that can be consumed by humans (which was almost certainly the primary original reason) and to eliminate the need for costly waste management programs (which has become very important in recent years as the world human population has increased and the amount of crop and food by-product has increased, particularly in developed countries). Ruminant feeding systems based on locally available by-product feedstuffs (BPF) are often a practical alternative because the rumen microbial ecosystem can utilize BPF, which often contain high levels of structural fibre, to meet their nutrient requirements for maintenance, growth, reproduction and production (Bampidis and Robinson, 2006; Mirzaei-Aghsaghali and Maheri-Sis, 2008a).

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely cultivated vegetable crops in Mediterranean countries. Significant amounts are consumed in the form of processed products such as tomato juice, paste, puree, ketchup and sauce. During tomato processing, a by-product known as tomato pomace (TP), is generated. Annual production of tomato pomace contains approximately 150000 metric ton (MT), in Iran. This by-product represents, at most, 4% of the fruit weight. Dried

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**Abbreviations: DTP,** Dried tomato pomace; **CP,** crude protein; **NDF,** neutral detergent fiber; **ADF,** acid detergent fiber; **NFC,** non fibrous carbohydrate; **OMD,** organic matter digestability; **ME,** metabolic energy; **SCFA,** short chain fatty acid; **NE₁,** net energy for lactation; **BPF,** by-product feedstuff; **TP,** tomato pomace; **DM,** dry matter.

tomato pomace contains 22.6 to 24.1% protein, 14.5 to 15.7% fat and 20.8 to 30.5% fiber. This by-product is a good source of vitamin $B_1$ and a reasonable source of vitamin A and $B_2$ (Aghajanzadeh-Golshani et al., 2010). Ziaei and Molaei (2010) demonstrated that tomato pomace silage containing 10% wheat straw can be advised for the sheep compared with alfalfa hay during the scarcity periods of roughages. Mahmoud Omar (2010) reported that tomato pomace can be used up to 30% of the concentrated diet during pregnancy and lactation of Awassi ewes. Ensiled wet tomato pomace used as supplement in dairy cows diet did not modify milk yield or its gross composition (Weiss et al., 1997). Bordowski and Geisman (1980) reported that tomato pomace seeds protein contains approximately 13% more lysine than soy protein. Abdollahzadeh et al. (2010) demonstrated that tomato pomace has the potential to be a good source of protein.

Three common methods including *in vivo, in situ* and *in vitro* have been used to evaluate the nutritive value of animal feedstuffs. The *in vitro gas* production technique has proved to be a potentially useful technique for feed evaluation, as it is capable of measuring rate and extent of nutrient degradation. In addition, *in vitro* gas production technique provide cost effective, easy to determine and suitable for use in developing countries. This method also can predicts feed intake (Khazaal et al., 1995), digestibility, microbial nitrogen supply, amount of short chain fatty acids, carbon dioxides and metabolizable energy of feeds for ruminants (Menke and Steingass, 1988; Babayemi, 2007; Mirzaei-Aghsaghali et al., 2008b; Maheri-Sis et al., 2008).

The objectives of this study were to determine the nutritive value of tomato pomace (DTP) including chemical composition, gas production characteristics, organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acids (SCFA) and net energy for lactation ($NE_l$) by *in vitro* gas production technique.

## MATERIALS AND METHODS

### Tomato pomace

Tomato pomace was obtained from "San San Shahd" factory in Urmia, Iran. The DTP was evaluated at the laboratories of Animal Science Research Institute, Karaj, Iran. Samples were collected, air-dried and ground (1 and 5 mm screen) for chemical analysis and *in vitro* gas production.

### Chemical analysis

Dry matter (DM) was determined by drying the samples at 105°C overnight and ash by igniting the samples in muffle furnace at 525°C for 8 h and nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude protein (CP) was calculated as N × 6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by procedures outlined by Georing and Van Soest (1970) with modifications described by Van Soest et al. (1991). Non fibrous carbohydrate (NFC) was calculated using the equation of NRC (2001); NFC% = 100 − (%NDF + %CP + % EE +

%Ash).

### *In vitro* gas production

*In vitro* gas production measurements were carried out in the laboratory of Animal Science Research Institute in Karaj. Fermentation of DTP samples were carried out with rumen fluid obtained from three mature canulated steers (age= 4.5 to 5 years; BW= 416 kg) fed twice daily with a diet (DMI= 8 kg/day) containing 70% hay (dry alfalfa and wheat straw with 70 to 30 ratio) and 30% concentrate (35% barley meal, 17% soybean meal, 25% whole cottonseed, 20% wheat bran, 1% $CaCO_3$ and 2% minerals and vitamins) as total mixed ratio, following the method described by Menke and Steingass (1988). Water was available *ad libitum*. The inoculum was prepared as described by Menke and Steingass (1988). It consisted of the rumen liquor mixed (1:2 v/v) with anaerobic artificial saliva. The latter included, for a final volume of 1 L, 237 ml of buffer solution, 237 ml of a main element solution, 0.12 ml of a trace element solution, 1.22 ml of resazurin solution (100 mg resazurin made up to 100 ml distilled water), 49.5 ml of a reduction solution (prepared fresh and separately and consisting of 2 ml of NaOH 1 N, 285 mg of $Na_2S$-$7H_2O$ and 47.5 ml distilled water for 1 L saliva), completed with 475 ml of distilled water. The buffer solution consisted of $NaHCO_3$ 35 g $l^{-1}$ and $NH_4HCO_3$ 4 g $l^{-1}$. The main element solution consisted of $Na_2HPO_4$ 5.70 g $l^{-1}$, $KH_2PO_4$ 6.20 g $l^{-1}$ and Mg $SO_4$-$7H_2O$ 0.60 g $l^{-1}$. The trace element solution consisted of $CaCl_2$-$2H_2O$ 13.20 g, $MnCl_2$-$4H_2O$ 10.00 g, $CoCl_2$-$6H_2O$ 1.00 g and $FeCl_2$-$6H_2O$ 0.80 g made up to 100 ml with distilled water. The rumen liquor was incorporated in the medium once the reduction process was achieved (resazurin decoloration after adding the reduction solution). All manipulations were done under continuous $CO_2$ reflux. Approximately, 200 mg DTP ground samples were weighed into the glass syringes of 100 ml. The fluid-buffer mixture (30 ml) was transferred into the glass syringes of 100 ml. The glass syringes containing DTP samples and rumen fluid-buffer mixture were incubated at 39°C. The syringes were gently shaken 30 min after the start of incubation. The gas production was determined at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h of incubation. All samples were incubated in triplicate with three syringes containing only rumen fluid-buffer mixture (blank). The net gas productions for DTP samples were determined by subtracting the volume of gas produced in the blanks (Menke and Steingass, 1988). Gas production data were fitted to the model of Ørskov and McDonald (1979).

$$Y = a + b\,(1 - e^{-ct}) \qquad (1)$$

Where, a is the gas production from the immediate soluble fraction (ml); b is the gas production from the immediately insoluble fraction (ml); c is the gas production rate constant for the insoluble fraction ($ml.h^{-1}$); a + b is the potential gas production (ml); t is the incubation time (h) and Y is the gas production at time t.

The ME (MJ $kg^{-1}$ DM) contents of DTP samples were calculated using equation of Menke and Steingass (1988) as follows:

$$ME\ (MJ\ kg^{-1}\ DM) = 2.20 + 0.136\ GP + 0.057\ CP \qquad (2)$$

Where, GP is the 24 h net gas production (ml $200^{-1}$ mg) and CP is the crude protein (%).

Organic matter digestibility (OMD) (%) of DTP samples were calculated using equation of Menke and Steingass (1988) as follows:

$$OMD\ (\%) = 14.88 + 0.889\ GP + 0.45\ CP + 0.0651\ XA \qquad (3)$$

Where, GP is the 24 h net gas production (ml $200^{-1}$ mg DM); CP is

**Table 1.** Chemical composition of tomato pomace.

| Component | % |
|---|---|
| DM | 96.30±0.04 |
| Crude protein (%DM) | 26.40±0.25 |
| Crude fiber (%DM) | 32.40±0.10 |
| Ether extract (%DM) | 15.90±0.08 |
| Ash (%DM) | 3.40±0.01 |
| NFC (%DM) | 3.70±0.18 |
| NDF (%DM) | 50.60±0.10 |
| ADF (%DM) | 34.50±0.41 |

NDF, Neutral detergent fiber; ADF, acid detergent fiber; NFC, non fibrous carbohydrate; calculated as 100 – (%NDF + %CP + % EE + %Ash) using the equation of NRC (2001).

the crude protein from the DTP sample (%) and XA is the ash content from the DTP sample (%).

Short chain fatty acid (SCFA) was calculated using the equation of Makkar (2005);

$$SCFA \text{ (mmol)} = 0.0222GP - 0.00425 \quad (4)$$

Where, GP is the 24 h net gas production (ml g$^{-1}$ DM).

NEl (MJ kg$^{-1}$ DM) = 0.115×GP + 0.0054×CP + 0.014×EE - 0.0054×CA - 0.36  (5)

Where, GP is the 24 h net gas production (ml 200$^{-1}$ mg DM), and CP, EE and CA are crude protein, ether extract, crude ash (%DM), respectively (Abas et al., 2005).

## RESULTS AND DISCUSSION

The chemical composition of DTP is shown in Table 1. The CP content in DTP was 26.4%. The CF, NDF, ADF and NFC contents were 32.4, 50.6, 34.5 and 3.7%, respectively.

The CP content of DTP in our study (26.4%) was higher than those reported by Ayhan and Aktan (2004) (17.32%) and Aghajanzadeh-Golshani et al. (2010) (22.7%). There are many factors that affect crude protein content such as stage of growth maturity and species or variety and soil types (Mirzaei-Aghsaghali et al., 2008b; Promkot and Wanapat, 2004). Those factors may partially explain the differences in crude protein content between our study and others. In addition, the EE, NDF, ADF contents were similar to that by Aghajanzadeh-Golshani et al. (2010). The ash content of by-product was lower than that reported by Ayhan and Aktan (2004) and Chumpawade (2009). The difference of ash content was probably due to variety of by-product or soil and sand contamination. The NFC content of DTP in our study (3.7%) was lower than that reported by Aghajanzadeh-Golshani et al. (2010) (6.63%). The NFC is used as sources of energy in dairy diets. The optimal dietary NFC in dairy diets is suggested to be between 30 to 40% dry

matter (DM) (Abdollahzadeh et al., 2010). There was a positive correlation between NFC content of feeds and gas production, but CP, NH$_3$-N and NDF contents were negatively correlated with gas production (Getachew et al., 2004; Maheri-Sis et al., 2007). This inconsistency may be due to tomato varieties, different methods of tomato processing, fruit maturity, management after harvest, growing conditions (and geographic and climatic conditions (Fontenont et al., 1977; Maheri-Sis et al., 2008). Different chemical composition leads to different nutritive value, because chemical composition is one of the most important indices of nutritive value of feeds. Variation in chemical components of feeds such as starch, NFC, OM, CP, NDF and soluble sugars contents can result in variation of *in vitro* gas production volume (Maheri-Sis et al., 2008).

Gas production volumes (ml 200$^{-1}$ mg DM) in different incubation times (Figure 1), gas production parameters (a, b, c) and calculated amounts of OMD, ME, SCFA and NEL of DTP are presented in Table 2. Gas volume at 24 h incubation (for 200 mg dry samples), soluble fraction (a), insoluble but fermentable fraction (b), potential gas production (a + b) and rate constant of gas production (c) were 39.38, -1.05, 52.96, 51.90 and 0.081 ml.h$^{-1}$, respectively.

Gas volume at 24 h incubation (for 200 mg dry samples) was similar to that by Aghajanzadeh-Golshani et al. (2010). Menke and Steingass (1988) suggested that gas volume at 24 h after incubation has relationship with metabolisable energy in feedstuffs. Sommart et al. (2000) reported that gas volume is a good parameter which predicts digestibility, fermentation end product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. Additionally, *in vitro* dry matter and organic matter digestibility were shown to have high correlation with gas volume (Sommart et al., 2000). Gas volumes also have shown a close relationship with feed intake (Blummel and Becker, 1997) and growth rate in cattle (Blummel and Ørskov, 1993).

The value for (a), intercept, in DTP was negative in this study. The negative (a) value for DTP due to delay in onset of fermentation and microbial attachment was in agreement with Aghajanzadeh-Golshani et al. (2010). The insoluble but fermentable fraction (b) of DTP in this study was higher than that reported by Aghajanzadeh-Golshani et al. (2010). The potential gas production (a + b) contents in our study were lower than those reported by Aghajanzadeh-Golshani et al. (2010), but was higher than that obtained by Besharati and Taghizadeh (2010). The rate constant of gas production (c) was lower than that reported by Aghajanzadeh-Golshani et al. (2010) and in line with Besharati and Taghizadeh (2010).

The OMD, ME, SCFA and NE$_l$ contents were 62 %, 9.06 MJ kg$^{-1}$ DM, 0.869 mmol and 4.51 MJ kg$^{-1}$ DM, respectively. The OMD and ME contents in this study were similar to the results of Aghajanzadeh-Golshani et al. (2010), while ME content was lower than those reported
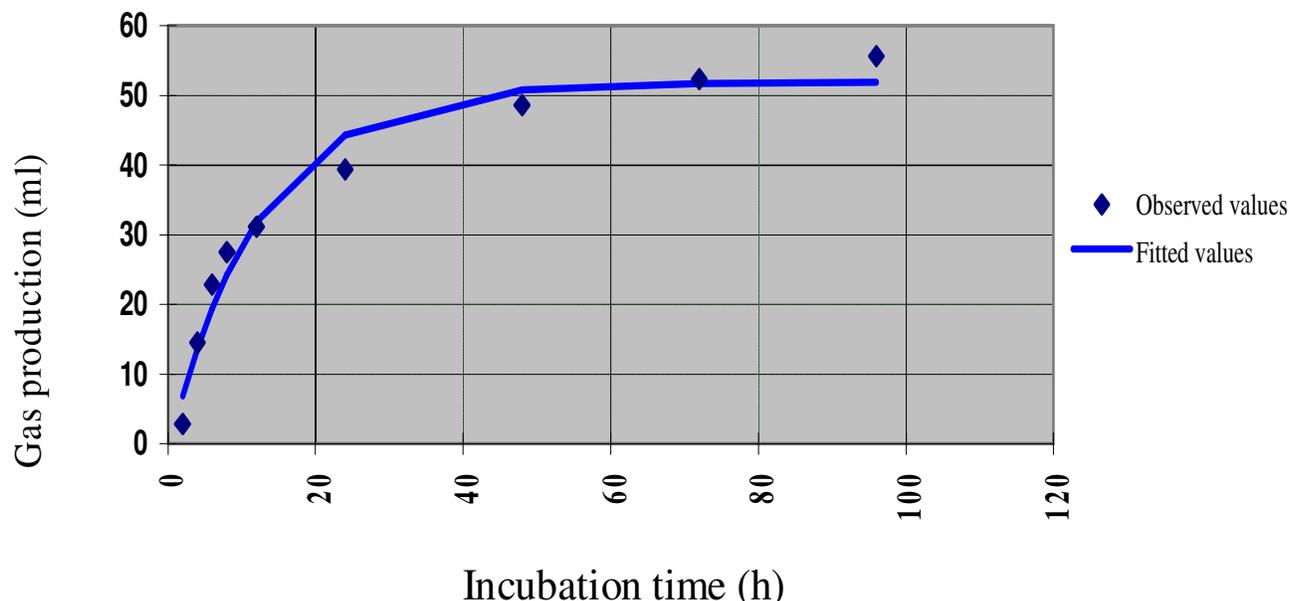
**Figure 1.** *In vitro* gas production volume of tomato pomace at different incubation time.

**Table 2.** *In vitro* gas production volume and estimated parameters of tomato pomace at different incubation times.

| Incubation time (h) | 2 | 4 | 6 | 8 | 12 | 24 | 48 | 72 | 96 |
|---|---|---|---|---|---|---|---|---|---|
| Gas production volume (ml) | 2.79 | 14.48 | 22.85 | 27.47 | 31.18 | 39.38 | 48.57 | 52.36 | 55.64 |
| | **a** | **b** | **(a+b)** | **c** | **OMD** | **ME** | **SCFA** | **NE$_l$** | |
| Estimated parameters | -1.05 | 52.96 | 51.90 | 0.081 | 62 | 9.06 | 0.869 | 4.51 | |

a = the gas production from the immediate soluble fraction (ml); b = the gas production from the immediately insoluble fraction (ml); c = the gas production rate constant for the insoluble fraction (ml/h); (a+b) = potential gas production (ml). OMD, Organic matter digestibility (% of DM); ME, metabolisable energy (MJ kg$^{-1}$ DM); SCFA, short chain fatty acid (mmol); NE$_l$, net energy for lactation (MJ kg$^{-1}$ DM).

by NRC (2001) and Besharati and Taghizadeh (2010). The NE$_l$ value was lower than that of reported by NRC (2001) and Weiss et al. (1997). Many factors such as differences in variety, environment conditions, method type and animal species may partially explain differences in OMD, ME and NE$_l$ contents between this study and others.

The SCFA content in this study was higher than the findings of Besharati and Taghizadeh (2010) and in line with Aghajanzadeh-Golshani et al. (2010). The SCFA contributes to at least 65 to 75% of the total metabolizable energy supply (Penner, et al., 2009). Gas volumes were produced quantitatively and qualitatively as a result of SCFA production (the amount of fermentative $CO_2$ and $CH_4$ could be accurately calculated from the amount and proportion of acetate, propionate and butyrate present in the incubation medium). Thus, increasing amount of SCFA was to increase gas production which resulted in high digestibility and energetic value (Maheri-Sis et al., 2008).

**Conclusion**

The results of this study based on chemical composition, OMD, ME, SCFA and NE$_l$ indicated that tomato pomace (DTP) could be a valuable food industrial by-product in ruminant nutrition. There is need for further *in vivo* study in order to claim these results.

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