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Full Length Research Paper

Estimation of the nutritive value of grape pomace for ruminant using gas production technique

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The aim of this study was to determine the chemical composition and estimation of nutritive value of white grape pomace (WGP) using *in vitro* gas production technique. Fermentation of WGP samples were carried out with rumen fluids obtained from three mature cannulated steers. The samples were collected from a factory in Urmia, Iran. The amount of gas production for WGP 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 17.27, 59.5, 52.5 and 13.5%, respectively. Gas production at 24 h and potential gas production (a + b) were 30.92 and 79.89 ml, respectively. The organic matter digestibility (OMD), metabolizable energy (ME) and short chain fatty acid (SCFA) contents were 50.50%, 7.4 MJ kg^{-1} DM and 0.69 mmol, respectively. The net energy for lactation (NEL) content was 3.31 MJ kg^{-1} DM. According to the results of this study, it seems that WGP could be used as a valuable food industrial by-product in ruminant nutrition.

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

INTRODUCTION

Iran faces scarcity in the quantity and quality of consistent year-round supplies of conventional ruminant feeds. Therefore, a better utilization of non-conventional feed resources, which do not compete as human foods, is imperative. Climatic conditions and shortage of water resources had increased costs of animal feeds in many countries (Alipour and Rouzbehan, 2006). The use of agricultural by-products obtained after processing of fruits, vegetables, crops and nuts, such as grape pomace, tomato pomace and brewers grain are often a useful way of overcoming this problem (Aghajanzadeh-Golshani et al., 2008; Bagheripour et al., 2008; Mirzaei-Aghsaghali and Maheri-Sis, 2008).

Annual production of grape pomace exceeds 50000 metric ton (MT), in Iran. Grape pomace consists mainly of peels, stems and seeds and accounts for about 20% of the weight of the grape processed into wine (Alipour and Rouzbehan, 2006; Brenes et al., 2008). Grape pomace has been used in diets of ruminants, fed close to maintenance metabolizable energy (ME) levels, especially in sheep (Abel and Icking, 1984). However, major limitations of using this by-product as a ruminant feed is the presence of a high level of lignified cell wall fraction and its high tannin content (Alipour and Rouzbehan, 2006; Abarghuei et al., 2010).

In vivo, situ and *vitro* methods have been used to evaluate the nutritive value of 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 is less expensive, easy to determine and suitable for use in

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Abbreviations: OMD, Organic matter digestibility, **ME,** metabolizable energy; **WGP,** white grape pomace; **CP,** crude protein; **NDF,** neutral detergent fiber; **ADF,** acid detergent fiber; **NFC,** non-fibrous carbohydrate; **OMD,** organic matter digestibility; **ME,** metabolizable energy; **SCFA,** short chain fatty acid; **NEL,** net energy for lactation; **DM,** dry matter.

Table 1. Chemical composition of white grape pomace.

Component	% Dry matter
DM	95.3 ± 0.01
Crude protein	17.27 ± 0.01
Crude fiber	22.8 ± 0.04
Ether extract	3.7 ± 0.00
Ash	5.7 ± 0.06
NFC	13.5 ± 0.67
NDF	59.5 ± 0.21
ADF	52.5 ± 5.85

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); DM, dry matter.

developing countries. This method also can be used to predict feed intake, digestibility, microbial nitrogen supply, amount of short chain fatty acids, carbon dioxide and metabolizable energy of feeds for ruminants (Menke and Steingass, 1988; Getachew et al., 2004; Babayemi, 2007; Maheri-Sis et al., 2008).

The objectives of this experiment were to determine the nutritive value of white grape pomace (WGP) including chemical composition, gas production characteristics, organic matter digestibility, metabolizable energy, net energy for lactation and short chain fatty acids by *in vitro* gas production technique.

MATERIALS AND METHODS

White grape pomace was obtained from a factory in Urmi, Iran. Following the extraction of juice, WGP 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 ashed by igniting the samples in muffle furnace at 525°C for 8 h. 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 Goering 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 WGP samples were carried out with rumen fluid obtained from three mature cannulated steers, fed twice daily with a diet containing hay (60%) and concentrate (40%) following the method described by Menke and Steingass (1988). Approximately 200 mg WGP 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 WGP 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 WGP samples were determined by subtracting the volume of gas produced in the blanks. Gas production data were fitted to the model of Ørskov and McDonald (1979):

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

Where, a = the gas production from the immediately 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); t = incubation time (h); Y = gas production at time t.

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

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

Where, GP = 24 h net gas production (ml 200^{-1} mg); CP = crude protein (%).

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

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

Where, GP = 24 h net gas production (ml 200^{-1} mg DM); CP = crude protein (%); XA = ash content (%).

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

$$SCFA\ (mmol) = 0.0222G - 0.00425.$$

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

NE_l (MJ kg^{-1} DM) = 0.115 × GP + 0.0054 × CP + 0.014 × EE - 0.0054 × CA - 0.36 (Abas et al., 2005).

Where, GP is 24 h net gas production (200 ml mg^{-1} DM) and CP, EE, CA and DOM are crude protein, ether extract, crude ash (% DM) and digestibility organic matter, respectively.

RESULTS AND DISCUSSION

The chemical composition of WGP is presented in Table 1. The CP content in WGP was 17.27%. The crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF) in WGP were 22.8, 59.5 and 52.5%; respectively. The NFC of WGP was 13.5%.

The CP, NDF and ADF contents of WGP in the study were higher than those reported by Pirmohammadi et al. (2007), Zalikaranab et al. (2007) and Bahrami (2010) but EE content was lower than those reported by Zalikaranab et al. (2007). This inconsistency may be due to grape pomace varieties, different methods of grape processing,

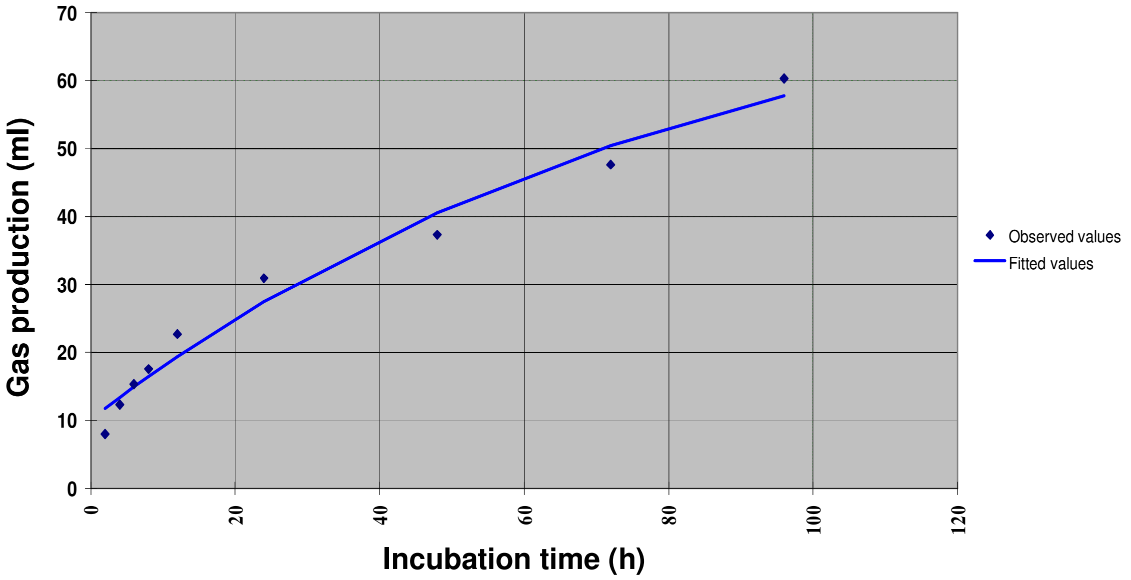

Figure 1. *In vitro* gas production volume of white grape pomace at different incubation time.

fruit maturity, management after harvest and growing conditions (including geographic/climatic conditions) (Fontenont et al., 1977; Maheri-Sis et al., 2008). Variations in chemical composition can lead 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 sugar contents can result in the variation of *in vitro* gas production volume (Maheri-Sis et al., 2008).

Gas production volumes (200 ml mg^{-1} DM) in different incubation times (Figure 1), gas production parameters (a, b and c) and calculated amounts of OMD, ME, SCFA and NEL of WGP 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 30.92 ml, 10.08 ml, 69.81 ml, 79.89 ml and 0.0119 ml/h, respectively.

Soluble fraction (a), insoluble but fermentable fraction (b), potential gas production (a + b) contents in the current study were higher than those reported by Alipour and Rouzbehan (2006) but the rate constant of gas production (c: 0.012) obtained in this work was lower than that (0.04) recorded by the same authors. Menke and Steingass (1988) suggested that gas volume at 24 h after incubation has a relationship with metabolisable energy in feedstuffs. Sommart et al. (2000) reported that gas volume is a good parameter to predict 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). There was a positive correlation between NFC content of feeds and gas production but feed CP, NH_3-N and NDF levels were negatively correlated with gas production (Getachew et al., 2004; Maheri-Sis et al., 2008).

The OMD, ME, SCFA and net energy for lactation (NEL) contents were 50.50% of DM, 7.4 MJ kg^{-1} DM, 0.69 mmol and 3.31 MJ kg^{-1} DM. The OMD and ME contents in the current study were higher than those reported by Pirmohammadi et al. (2007) (37.18% and 5.48 MJ kg^{-1} DM) and Alipour and Rouzbehan (2006) (44.19% and 6.69 MJ kg^{-1} DM). The different results reported by Pirmohammadi et al. (2007) and Alipour and Rouzbehan (2006) about OMD and ME may be due to differences in variety, environmental conditions and animal species.

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 led to increase in gas production which also resulted in high digestibility and energetic value (Maheri-Sis et al., 2008).

Conclusion

In view of the worldwide demand for additional feed sources, the exploitation of traditional crops, which often are grown with low inputs, and are largely adapted to the

Table 2. *In vitro* gas production volume and estimated parameters (mean ± SD) of white grape pomace at different incubation times.

Incubation time (h)	**2**	**4**	**6**	**8**	**12**	**24**	**48**	**72**	**96**
Gas production volume (ml)	8 ± 0.31	12.33±0.53	15.33±0.70	17.57±0.56	22.7±1.17	30.92±1.52	37.33±2.05	47.63±1.93	60.30±2.36
Estimated parameters	**a**	**B**	**(a+b)**	**c**	**OMD**	**ME**	**SCFA**	**NE_l**	
	10.08±1.33	69.81±2.70	79.89±2.14	0.0119±0.001	50.50±1.43	7.40±0.25	0.69±0.05	3.31±0.11	

a , The gas production from the immediately 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, metabolizable energy (MJ kg^{-1} DM); SCFA, short chain fatty acid (mmol); NE_l, net energy for lactation (MJ kg^{-1} DM).

climatic conditions of the developing countries, would be a step towards better resource utilization. The results of the current study based on chemical composition, OMD, ME, SCFA and NEL indicated that white grape pomace can be used as valuable feedstuffs in ruminant's nutrition. Further studies on nutritive value of white grape pomace using *in vivo* technique may prove useful in further evaluation of the feed value of this type of grape pomace.

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