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Evaluating nutritional value of apple pomace for ruminants using *in vitro* gas production technique

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ABSTRACT

This study was carried out to determine the chemical composition and estimation of nutritive value of apple pomace (AP) by-product using in vitro gas production technique. Fermentation of AP samples were carried out with rumen fluid obtained from three mature canulated steers. The samples were collected from SanSan Shahd factory in Urmia, Iran. The amount of gas production for AP at 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours were measured. The results showed that the crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE), ash and non-fibrous carbohydrate (NFC) contents were 90.2, 612, 467, 37, 23 and 237.8 g/kg DM, respectively. Gas volume at 24 h incubation (for 200 mg dry sample), soluble fraction (a), insoluble but fermentable fraction (b), potential gas production (a + b) and rate constant of gas production (c) contents were 58.92, 0.9095, 76.91 and 77.82 ml/200mg DM and 0.0574 ml/h, respectively. Calculated amounts of OMD, ME, SCFA and NE_l *were 714.6 g/kg DM, 10.73 MJ/kg DM, 1.304 mmol and 6.504 MJ/kg DM, respectively. According to results of this study it seems that AP could be used as a valuable by-product in ruminant nutrition.*

Key words: nutritive value, digestibility, canulated steers, *in vitro* gas production, by-product, apple pomace, short chain fatty acid, net energy for lactation.

Abbreviations: *AP, apple pomace; EAP, ensiled apple 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,* NE_l*, net energy for lactation; PBF, by-product feedstuffs; DM, dry matter; EE, ether extract; BW, body weight; IVDMD, in vitro dry matter digestibility.*

INTRODUCTION

Nowadays, there is great political and social pressure to reduce the pollution arising from industrial activities. Almost all developed and underdeveloped countries are trying to adapt to this reality by modifying their processes so that their residues can be recycled. Consequently, most large companies no longer consider residues as waste, but as a raw material for other processes. Most by-product feedstuffs (BPF) result from the processing of commercial crops, the food processing industry and the fiber industry.

Many agricultural and agro-industrial by-products that could profitably be used are available locally but are no fully exploited for the feeding of livestock. Such feedstuffs include fibrous by-products such as wheat bran, maize bran, rice bran, tomato pomace, grape pomace, sugar beet pulp, pomegranate pulp and apple pomace. This agro-industrial by products, although containing potentially toxic components, can be improved by various treatments such as chemical, mechanical, pelleting, grinding and other processing techniques. Many by-products have a substantial potential value as animal feedstuffs. The utilization of agro-industrial by products may be economically worthwhile, since conventional feedstuffs are often expensive. Several factors have lead to increase interest in by-product feedstuffs, such as pollution abatement and regulations, increasing costs of waste disposal and changes in perception of the value of by-product feedstuffs as economical feed alternatives [1,2].

Apple pomace, a by-product of juice or puree making industry, is a rich source of many nutrients including carbohydrates, minerals, except protein, and in Iran, production of this by-product exceeds 97,000 t/year. Apple pomace has been utilized as animal feed after ensiling or after drying [1,3]. Rumsey and Lindahl showed that a diet consisting primarily of urea-supplemented AP adversely affects weight gain and lambing performance of ewes [4], as reported previously with cows [5]. Furthermore, the adverse effects can be diminished by the inclusion of straw in the diet, as shown previously with cows [6]. Unlike gestating cows, an adequate nutrient intake by gestating ewes is not possible when AP is the primary diet ingredient, regardless of the type of protein supplement. One study has indicated that, increased milk production, milk fat and decreased feeding cost when AP was mixed well with 10% wheat barn, 10% chopped alfalfa and 10% milled rice barn and were ensiled then fed to Holstein dairy cow [7]. Another study has indicated that, ensiled apple pomace (EAP) can substitute successfully at diets up to 30% without negative effect on milk yield and milk composition (fat, protein and SNF) [8]. Kafilzadeh et al., [3] showed that AP from puree making had higher digestibility than the AP from juice making.

In vitro methods of feed evaluation have numerous advantages over other methods (such as *in vivo*, *in sacco*). They are less expensive, less time-consuming and allow incubation conditions to be maintained more precisely than *in vivo*. In addition, in vitro techniques utilize small amounts of test feeds making them applicable to screening of feeds that are not available in sufficient quantity for *in vivo* experiments. The *in vitro* method of Tilley and Terry [9], *in sacco* method of Mehrez and Ørskov [10], and enzymatic method of Jones and Hayward [11] have all been widely used to predict digestibility of feeds, and used as a selection tool for screening feeds for nutritional quality. Menke and Steingass [12] reported a strong correlation between metabolizable energy (ME) values measured *in vivo* and predicted from 24 h *in vitro* gas production and chemical composition of feeds. The *in vitro* gas production method has also been widely used to evaluate the energy value of several classes of feeds, particularly straws [13], agro-industrial by-products [14], compound feeds [15] and various tropical feeds [16]. The technique has also been used to assess effects of anti-nutritive factors on rumen fermentation [17]. However, this technique is measuring gas produced by the fermentation of energy containing components in feeds, and not only that of protein [18, 19, 20, 21].

Therefore, the objective of this study is to assess the nutritional composition of AP by its chemical composition, *in vitro* fermentation characteristics, organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acids (SCFA) and net energy for lactation (NE_l).

MATERIALS AND METHODS

2.1. Apple pomace

Randomly fresh AP samples were collected from the SanSan Shahd factory in Uromia (Iran). Samples air-dried and ground (1mm and 5mm screen) for chemical analysis and *in vitro* gas production, and evaluated at the laboratories of Animal Science Research Institute in Karaj.

2.2. Chemical Analysis

Dry matter (DM) was determined by drying the samples at 105 ^{o}C overnight and ash by igniting the samples in muffle furnace at 525 ^{o}C for 8h and Nitrogen (N) content was measured by the Kjeldahl method [22]. 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 [23] with modifications described by Van Soest et al. [24]. Non-Fibrous Carbohydrate (NFC) is calculated using the equation of NRC [25], NFC = 100 – (NDF + CP + EE + Ash).

2.3. In vitro gas production

Fermentation of AP samples were carried out with rumen fluid obtained from three mature canulated steers (BW=550 kg) fed twice daily a diet (DMI= 12.37 kg/day) containing lucerne hay (600 g/kg) plus concentrate mixture (400 g/kg) following the method described by Menke and Steingass [12]. Both solid and liquid rumen fractions were collected before the morning feeding, placed in an insulated plastic container, sealed immediately and transported to the laboratory. Approximately 200 mg AP 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 samples and rumen fluid-buffer mixture were incubated at 39 ^{o}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 AP 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 [26].

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

Where P is the gas production at time t, a the gas production from soluble fraction (ml/200mg DM), b the gas production from insoluble fraction (ml/200mg DM), c the gas production rate constant (ml/h), $a + b$ the potential gas production (ml/200mg DM) and t is the incubation time (h).

The ME and OMD contents of AP by-product were calculated using equations of Menke and Steingass [12] as

ME (MJ /kg DM) = 2.20 + 0.136 × GP + 0.057 × CP
OMD (g/kg DM) = 14.88 + 0.889 × GP + 0.45 × CP + 0.0651 × XA

GP = 24 h net gas production (ml/200mg).
CP = Crude protein (g/kg DM)
XA = Ash content (g/kg DM)

Short chain fatty acids (SCFA) is calculated using the equation of Makkar [27],
Where, Gas is 24 h net gas production (ml/200mg DM).

SCFA (mmol) = 0.0222 × GP – 0.00425

NEL (MJ/kg DM) = 0.115 × GP + 0.0054 × CP + 0.014 × EE - 0.0054 × CA - 0.36 [28].

Where, GP is 24 h net gas production (ml/200 mg DM), and CP, EE, CA and DOM are crude protein, ether extract, crude ash and digestibility organic matter (g/kg DM), respectively.

In vitro gas production measurements were carried out in the laboratory of Animal Science Research Institute in Karaj.

RESULTS

3.1. Chemical composition

The chemical composition data of AP by-product are presented in Table 1. The CP concentration was 150 ± 0.16 g/kg DM. The NDF and ADF contents were 612 ± 0.2 and 467 ± 0.15 g/kg DM, respectively, whereas the NFC content was 237.8 ± 4.6 g/kg DM. The EE and ash contents were 37 ± 0.27 and 23 ± 0.25 g/kg DM, respectively.

Table 1 Chemical composition of apple pomace (g/kg DM, except DM g/kg fresh base)

DM	CP	NDF	ADF	EE	Ash	NFC
952 ± 0. 12	90.2 ± 0.16	612 ± 0.2	467 ± 0.15	37 ± 0.27	23 ± 0.25	237.8 ± 4.6

DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, EE: ether extract, NFC: non-fiber carbohydrate.

Table 2 *In vitro* gas production volumes (ml/200mg DM) and estimated parameters of apple pomace (AP) at different incubation times

Time (h)	**2**	**4**	**6**	**8**	**12**	**24**	**48**	**72**	**96**
Gas volume (ml)	8.67	15.5	21.83	30.57	43.5	58.92	65.17	74.16	85.05
Estimated parameters[a]									
	a	*b*	(*a* + *b*)	*c*	OMD	ME	SCFA	NE_l	
AP	0.9095	76.91	77.82	0.0574	714.6	10.73	1.304	6.504	

[a] a: the gas production from soluble fraction (ml/200mg DM), b: the gas production from insoluble fraction (ml/200mg DM), c: rate constant of gas production during incubation (ml/h), (a + b): the potential gas production (ml/200mg DM), OMD: Organic matter digestibility (g/kg DM), ME: Metabolisable energy (MJ/kg DM), SCFA: Short chain fatty acid (mmol), NE_l: net energy lactation (MJ/kg DM).

3.2. In vitro gas production

Gas production volumes (ml/200mg DM) in different incubation times (Fig. 1), gas production parameters (*a*, *b*, *c*) and calculated amounts of OMD, ME, SCFA and NE_l of AP 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 58.92, 0.9095, 76.91, 77.82 ml/200mg DM and 0.0574 ml/h, respectively. Calculated amounts of OMD, ME, SCFA and NE_l were 714.6 g/kg DM, 10.73 MJ/kg DM, 1.304 mmol and 6.504 MJ/kg DM, respectively.

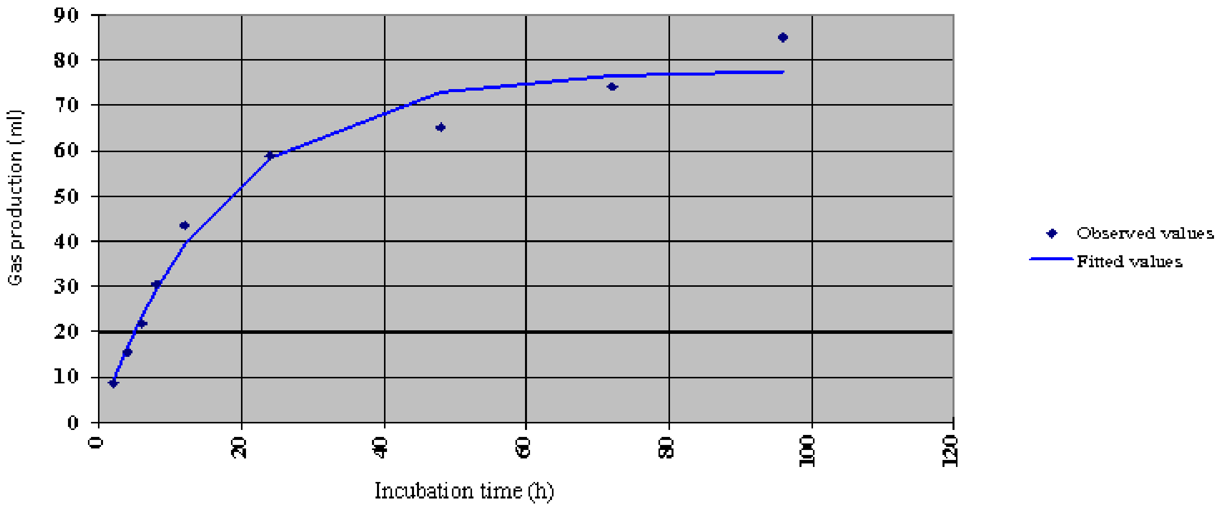

Fig. 1. *In vitro* gas production volume of apple pomace at different incubation time.

DISCUSSION

4.1. *Chemical composition*

The crude protein content (90.2 g/kg DM) was higher than those reported by Taasoli and Kafilzadeh [29] (51.2 g/kg DM) and Besharati and taghizadeh [1] (52.5 g/kg DM). Singhal et al., [30] in a study feeding dried AP reported lower values for NDF (300 g/kg DM) and ADF (250 g/kg DM) than those observed in the present study (612 and 467 g/kg DM, respectively). Also, NDF and ADF contents in the current study were about 1.73 and 1.66 times higher (612 and 467 vs. 353 and 280 g/kg DM, respectively) than that reported by Besharati and taghizadeh [1]. Pirmohammadi et al., [31] found lower NDF (463 g/kg DM) and higher ADF (405 g/kg DM) in dried AP. The ether extract (EE) of AP was 37 g/kg DM, a value lower than that of 61.75 g/kg DM reported by Taasoli and Kafilzadeh [29] and in line with Besharati and taghizadeh [1] (37 g/kg DM). Taasoli and Kafilzadeh [29] in a study feeding dried AP reported higher values for ash (37 g/kg DM) and NFC (464.40 g/kg DM) than those observed in the present study (23 and 237.8 g/kg DM, respectively). Such difference in the chemical composition of AP can be expected due to the morphology of the original apple, the extraction technique [32] and probably drying method used.

4.2. In vitro gas production

Gas production volumes (ml/200 mg DM) in different incubation times (Fig. 1), gas production parameters (a, b, c) and calculated amounts of SCFA, OMD, ME and NE_l of AP are presented in Table 2. Gas volume at 24 h incubation (for 200 mg dry samples) was about 2.23 times higher than that reported Besharati and taghizadeh [1]. Cumulative gas volume at each sampling time was affected by variety of feedstuffs. These finding indicate that fraction of substrate and degradability of AP are difference. Sommart et al. [33] reported that gas volume is a good parameter from which to predict digestibility, fermentation end product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. The potential gas production (*a* + *b*) value in the current study was higher than that reported by Besharati and taghizadeh [1], while rate constant of gas production (*c*) content was lower. High rate of gas production (0.084 vs. 0.057 ml/h) possibly influenced by carbohydrate fractions readily availability to the microbial population.

The OMD content was 714.6 g/kg DM, a value higher than that of 643 g/kg DM reported by Besharati and taghizadeh [1], while a value lower than that of 721.7 reported by Taasoli and Kafilzadeh [29]. The different result reported by Besharati and Taghizadeh [1] and Taasoli and Kafilzadeh [29] about OMD may be due to differences in variety, environment conditions, concentration of cell wall contents. *In vitro* dry matter and organic matter digestibility were shown to have high correlation with gas volume [33]. The ME value in the present study was higher than reported by Givens and Barber [34], NRC [25] and Besharati and Taghizadeh [1]. Menke and Steingass [12] suggested that gas volume at 24 h after incubation has been relationship with metabolisable energy in feedstuffs. Apple pomacel is of high energetic value as compared to elected feedstuffs commonly fed to cattle [25], such as alfalfa (ME 8.20 MJ/kg DM), almond hulls (ME 7.91 MJ/kg DM), barely grain (ME 12.22 MJ/kg DM), barely silage (ME 8.49 MJ/kg DM), citrus pulp (ME 11.54 MJ/kg DM), corn silage (ME 9.74 MJ/kg DM), sorghum silage (ME 7.49 MJ/kg DM), and tomato pomace (ME 9.91 MJ/kg DM). It was also illustrated by the negative correlation between ME, OMD and *in vitro* dry matter digestibility (IVDMD) and the ADF content (r ranging from -0.79 to -0.99) [35]. Besharati and Taghizadeh [1] in a study feeding dried AP reported lower values for SCFAs (1.168 mmol) than that observed in the current study (1.304 mmol). Ruminants depend on SCFAs for up to 80% of their maintenance energy requirements. Getachew et al. [19] reported the close association between SCFA and gas production *in vitro*, and the use of this relationship between SCFA and gas production to estimate the SCFA from gas values, which is an indicator of energy availability to the animal. The NE_l value of AP was higher than that reported by NRC [25]. The AP is of good NE_l value as compared to elected feedstuffs commonly fed to cattle [25], such as alfalfa (NE_l 6.36 MJ/kg DM), almond hulls (NE_l 4.77 MJ/kg DM), barely grain (NE_l 7.78 MJ/kg DM), citrus pulp (NE_l 7.36 MJ/kg DM) and tomato pomace (NE_l 6.36 MJ/kg DM).

CONCLUSION

The results of current study based on chemical composition, in vitro gas production parameters, and OMD, ME, SCFA and NE_l content of apple pomace indicated that it could be as a valuable food industrial by-product in ruminant nutrition.

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