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Evaluation of some by-Products using *In situ* and *In vitro* Gas Production Techniques

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Abstract: Food by-products in Iran are produced in high levels. In this study, *in situ* and *in vitro* gas production techniques were used to describe nutritive value of apple pomace, tomato pomace and noodle waste. For this purpose two ruminal fistulated sheep were used. Nylon bags which were approximately (6×12 cm) containing 5 g samples (2 mm screen) were incubated in duplicate in the rumen of fistulated sheep for 0,2,4,6,8,12,16,24,36 and 48 h. The gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36 and 48 h of incubation and the equation of $P = A (1-e^{-ct})$ was used to describe the kinetics of gas production. The data was analyzed using completely randomized design. DM and CP disappearance were significantly different among feedstuffs ($p<0.05$). After 48 h of incubation DM disappearance in noodle waste was highest and in tomato pomace was lowest. Regarding to the results, at the most incubation times tomato pomace had lower CP disappearance among feedstuffs ($p<0.05$). Potential gas production (A) and rates of gas production (c) differed among feedstuffs. Apple pomace showed higher potential gas production (A) (305.1 mL g^{-1} DM) and tomato pomace had higher rate of gas production (c) (0.09 h^{-1}) than the other feedstuffs. According to gas production volume, the value for the ME, OMD and SCFA ranged from in 8.87 noodle waste to 9.76 in apple pomace, 56.1 in tomato pomace to 64.3 in apple pomace and 0.919 in noodle waste to 1.168 in apple pomace, respectively. Partitioning factor in noodle waste was highest and in tomato pomace was lowest. In the present study, feeds composition significantly affected the degradation parameters.

Key words: By-product, *in situ*, gas production, metabolizable energy

INTRODUCTION

A major constraint to increasing livestock productivity in developing countries is the scarcity and fluctuating quantity and quality of the year-round supply of conventional feeds. These countries experience serious shortages in animal feeds of the conventional type. In order to meet the projected high demand of livestock products and to fulfill the future hopes of feeding the millions and safeguarding their food security, the better utilization of non-conventional feed resources which do not compete with human food is imperative. There is also a need to identify and introduce new and lesser known food and feed crops. An important class of non-conventional feeds is by-product feedstuffs which are obtained during harvesting or processing of a commodity in which human food or fibre is derived. The amount of by-product feedstuffs generally increases as the human population increases and economies grow.

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.

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Large quantities of by-products are used in the ruminant's diet in agro-industrial areas[1]. In Iran, animals suffer from under feeding and mal-nutrition in winter due to the shortage of local produced feed which are not sufficient to cover the nutritional requirements of animals. The annually amount produced of agro-by-products in Iran are generous. In Iran, production of apple pomace, tomato pomace and noodle waste exceeds 97000, 150000 and 65000 ton/year, respectively. The potential use of these wastes in

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ruminant ration will participate in reducing the shortage of feedstuffs and subsequently increase milk and meat production in Iran. However, little is known about their fermentation pattern in the rumen and a better understanding of their digestion and products of fermentation is necessary in order to properly balance their introduction into the diets[2,3] and the knowledge about their potential feeding value is insufficient. The nutrient composition of feeds is commonly determined by chemical analysis. However, this does not provide sufficient information to determining the feed true nutritive value.

There is little information available on the nutritive value of Apple Pomace (AP), Tomato Pomace (TP) and Noodle Waste (NW) produced in Iran. The present study was, therefore, carried out to determine the chemical composition, digestibility and degradability of AP, TP and NW. There are several methods to evaluate feedstuffs. In this study, *in situ* and *in vitro* gas production techniques were used to describe nutritive value of grape by-products.

MATERIALS AND METHODS

Preparing of by-product: AP, TP and NW were obtained from raisin production factories of Tabriz, Iran.

Chemical composition: The chemical composition of AP, TP and NW were determined using the methods recommended by AOAC[4]. NDF and ADF were determined using the methods of Van Soest *et al*.[5].

In Sacco degradation trial: Rumen degradation characteristics of feeds[6] were calculated after the incubation 5 g samples of AP, TP and NW (ground at 2 mm) in nylon bags. The bag size was 12×6 cm with a pore size of 50 μm. Bags were incubated in the rumen of two cannulated sheep for 0, 2, 4, 6, 8, 12, 16, 24, 36 and 48 h. The animals were offered 400 g lucerne, 200 g barley and 200 g soybean meal twice a day at 09:00 h and 17:00 h (NRC, 1980)[7]. Animals had free access to water. After removal, bags were washed under tap water for at least 15 min. After thorough hand squeezing, bags were dried for 24 h at 65°C and 24 h at 105°C then weighed. Feed residues were recovered from each bag and stored pending analysis for Kjeldahl nitrogen. The value of degradability at time 0 h was obtained by washing four bags under tap water for at least 15 min. For each bag, the residue was analysed for DM and nitrogen. The percentage of degradability (Y) of DM and nitrogen at time (t) was obtained from an exponential equation:

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

which was fitted to the experimental data by iterative regression analysis[8]. In this equation, e is the base of natural logarithms, the constant 'a' represents the soluble and very rapidly degradable component and 'b' represents the insoluble but potentially degradable component, which degrades at a constant fractional rate (c) per unit time. The effective degradability of DM and CP for feedstuffs was then estimated by the following equation:

$$\text{Effective degradability } (\%) = a + bc/(c + k)$$

In this equation, k refers to the fractional outflow rate of small particles from the rumen. A value of 0.02 fraction/h for k was used.

***In vitro* gas production trial:** The dry matter degradability of each by-product was determined by *in vitro* fermentation with ruminal fluid. Ruminal fluid was collected approximately 2 h after morning feeding from two cannulated sheep consuming 400 g alfalfa hay, 300 g barley and 300 g soybean meal. Ruminal fluid was immediately squeezed through four layers of cheesecloth and was transported to the laboratory in a sealed thermos. The resulting ruminal fluid was purged with deoxygenated CO_2 before use as the inoculum. Gas production was measured by Fedorak and Hrudy[9] method. Approximately 300 mg of dried and ground (2 mm) by-products samples were weighed and placed into serum bottles. Buffered rumen fluid with McDougal buffer (20 mL) was pipetted into each serum bottle[10]. The gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36 and 48 h of incubation. Total gas values were corrected for the blank incubation and reported gas values are expressed in mL 1 g^{-1} of DM. The gas production profiles in triplicate fitted with equation of $Y = A\ (1\text{-}e^{-ct})$ where Y is the volume of gas production (mL g^{-1} DM) at time t, A is gas production from soluble and insoluble fraction, c is the gas production rate and t is the incubation time (h). The Metabolizable Energy (ME) contents of GP and OMD were calculated using equations of Menke *et al*.[11] as:

$$ME_{(MJ\,kg^{-1}\,DM)} = 2.20 + 0.136\times GP + 0.057\times CP + 0.0029\times CP^2$$

$$OMD_{(g\,100\,g^{-1}\,DM)} = 14.88 + 0.889\times GP + 0.45\times CP + 0.0651\times XA$$

where, XA ash in g 100 g^{-1} DM and GP is the net gas production (mL) at 24 h. The short chain fatty acid was calculated using blow equation as:

$$SCFA_{(mmoL)} = -0.00425+0.0222GP$$

where,Gas is 24 h net gas production (mL g^{-1} DM).

Partitioning factor (PF): In a separate run of gas production completed on by-products samples, the method of Blummel *et al*.[12] was adopted to determine the Partitioning Factor (PF). In this method, approximately 300 mg of dried and ground (2mm) by-products samples were weighted and placed into serum bottles. Buffered rumen fluid with McDougal buffer (20ml) was pipetted into each serum bottle to measure gas production to 24 h. After termination of incubation, bottles contents were transferred to tubes and centrifuged at 2500×g for 10 min. Supernatants were picked out and solid parts were washed with buffer (NaHPO3, KHPO3, NaCl and distilled water) and centrifuged at 2500×g for 10 min. Then the solid parts were dried in the oven and weighed. The ratio of DM truly degraded (mg) to gas volume at 24 h incubation was used as the partitioning factor[12].

Statistical analysis: Data obtained from *in situ* and gas production studies was subjected to analysis of variance as a completely randomized design by the GLM procedure of SAS Institute Inc[13] and treatment means were compared by the Duncan test.

RESULTS AND DISCUSSION

The chemical compositions of feedstuffs are shown in Table 1. The CP of AP, TP and NW were 5.25, 21.59 and 11.27%, respectively. ADF, NDF, CF and ASH contents in AP were 28, 35.3, 3.7 and 2.2%, respectively. For TP the contents of ADF, NDF, CF and ASH were 58.7, 67.4, 6.9 and 12.2%, respectively.

In situ DM disappearance of feedstuffs at the incubations times are given in Table 2. There were differences among levels of disappearance for DM of feedstuffs at the different incubation times ($p<0.05$). At the time 0 h of incubation, AP had higher DM disappearance than the other feedstuffs ($p<0.05$). After 48 h of incubation DM disappearance in NW was greater than other test feeds ($p<0.05$).

Rapidly degraded fraction (a) for AP was higher than the other feedstuffs, which it shows the AP has high level of non structural carbohydrates. NW had greatest amount of fraction b (insoluble but fermentable component) among feedstuffs. The lower fraction a in TP may be caused by higher level of NDF in TP (Table 1). The dry matter ED of NW was higher than the other feedstuffs, this may be because of its lower NDF and higher fermentable fraction.

Pirmohammadi *et al*.[14] reported a, b, c fractions and dry matter ED for AP 38.5, 51.3, 0.062 and 67.4, respectively, that there was slightly difference with present study. This difference may be because of present of difference in species of apple, manner of expressed juice and preparing of AP. The pattern of DM disappearance of test feeds was distinctly different (Fig. 1). Noodle waste degraded faster and tomato pomace degraded slower than the other test feeds ($p<0.05$).

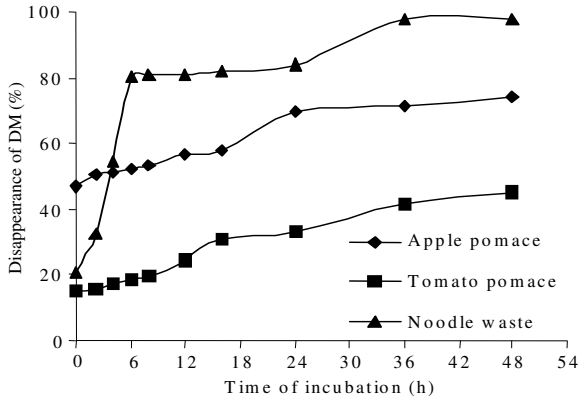

Fig. 1: Pattern of *in situ* DM disappearance of test feeds

Table 1: The chemical composition of feeds (%DM)

Feeds	DM	CP	NDF	ADF	EE	OM
Apple pomace	92.34	5.25	35.3	28	3.7	97.8
Tomato pomace	96.48	21.59	67.4	58.7	6.9	87.8
Noodle waste	90.77	11.27	-	-	0.7	98.8

Table 2: *In situ* disappearance of DM (%)*

	Time of incubation (h)													
Feeds	0	2	4	6	8	12	16	24	36	48	a[1]	b[2]	c[3]	ED[4]
AP[5]	46.9[a]	50.3[a]	50.8[b]	51.9[b]	53[b]	56.5[b]	57.8[b]	69.2[b]	71.1[b]	74.2[b]	46.7	40.1	0.026	69.3
TP[6]	15.1[c]	15.6[c]	17.6[c]	18.3[c]	19.9[c]	24.5[c]	30.6[c]	32.9[c]	41.6[c]	45.3[c]	13.6	51.1	0.021	39.7
NW[7]	20.7[b]	32.6[b]	54.4[a]	80.4[a]	80.7[a]	80.8[a]	81.8[a]	84[a]	97.6[a]	97.7[a]	17.1	75.1	0.195	85.2
SEM	0.23	2.37	1.04	1.62	2.74	2.11	0.94	0.97	0.76	0.96	-	-	-	-

*: The means within a column without common letter(s) differ ($p<0.05$). 1: Rapidly degraded fraction (%); 2: Slowly degraded fraction (%); 3: Rate of degradation (fraction/h); 4: Effective degradability (%)(out flow rate: 0.02 h); 5: Apple pomace; 6: Tomato pomace; 7: Noodle waste

Table 3: *In situ* disappearance of CP (%)*

	Time of incubation (h)													
Feeds	0	2	4	6	8	12	16	24	36	48	a[1]	b[2]	c[3]	ED[4]
AP	38.5[b]	41.5[b]	45.8[b]	46.7[b]	46.8[b]	47[c]	51.8[c]	78.2[b]	81.5[b]	86.8[b]	35.6	64.4	0.306	74.5
TP	16.7[c]	22.5[c]	31.2[c]	36.2[c]	42.5[c]	55.4[b]	58.8[b]	60.6[c]	64.5[c]	68.2[c]	14.6	53	0.099	58.7
NW	67.7[a]	85.3[a]	86.4[a]	89.4[a]	91.8[a]	93.2[a]	94.2[a]	95.5[a]	97.2[a]	98.2[a]	69.3	26.3	0.294	93.9
SEM	1.05	1.25	0.86	0.82	1.31	0.95	0.56	1.42	1.16	1.14	-	-	-	-

*: The means within a column without common letter(s) differ (p<0.05). 1: Rapidly degraded fraction (%); 2: Slowly degraded fraction (%); 3: Rate of degradation (fraction/h); 4: Effective degradability (%)(out flow rate: 0.02 h)

Table 4: Gas production of feedstuffs incubated in buffered rumen fluid (mL g^{-1} DM)*

	Time of incubation (h)								
Feeds	2	4	6	8	12	16	24	36	48
AP	37.2[a]	94.4[a]	136.1[a]	164.7[a]	201.9[a]	230.2[a]	264.2[a]	295.1[a]	305.1[a]
TP	31.4[a]	63.7[b]	84.6[c]	104.6[c]	131.6[c]	151.7[c]	172.4[c]	191.8[c]	197.2[c]
NW	31.2[a]	70.8[b]	95[b]	124.1[b]	160.7[b]	180.6[b]	208[b]	237.1[b]	245.6[b]
SEM	1.68	2.22	2.98	2.33	7.44	7.88	7.53	7.06	8.02

*: The means within a column without common letter(s) differ (p<0.05)

In situ CP disappearance of feedstuffs at the incubations times are shown in Table 3. Among feedstuffs, NW had higher CP disappearance at time 0 h (p<0.05). At the most incubation times TP had lower CP disappearance among feedstuffs (p<0.05). The soluble CP fraction (a) for NW was higher than the other feedstuffs and TP had lowest fraction a among feedstuffs. The insoluble but fermentable component (fraction b) and fraction c in AP were greater than the other feedstuffs (p<0.05). Protein degradation in the feedstuffs had a similar trend to that of DM degradation. Limited information is available on the degradability of these feedstuffs. Pirmohammadi *et al*.[14] reported a, b and c fractions and crude protein ED for AP 40.1, 56.5, 0.066 and 72.1, respectively, that there was slightly difference with present study.

The mean values of the a, b and c fractions of protein in fresh AP reported by NRC[15] were 41.7, 53.3 and 0.05% h, respectively. This compares well with the corresponding values obtained for AP in this study. However, in low protein feeds, the residues in the Dacron bag may be contaminated with microbial protein and this will lead to an apparently lower degradability of feed protein[16]. NRC[15] reported the amounts of a, b and c fractions for TP 41.7, 53.3 and 0.05% h, respectively. There was difference between amounts reported by NRC[15] and this study.

The volumes of gas production of feedstuffs are shown in Table 4. There were significantly differences in gas production volumes among feedstuffs at different incubation times (p<0.05). After 48 h incubation TP had lowest gas production volume among feedstuffs (p<0.05). Low gas yield for TP in initial incubation times compared to the other test feeds was resulted due to high content of slowly fermented carbohydrates in TP.TP had high level of NDF and it needs more time to attachment of microorganism. The high level of AP and NW gas yield can be assumed that degradable nitrogen was not limiting microbial activity allowing the AP and NW carbohydrate fractions be degraded according to their potential. Gasmi-Boubaker *et al.*[17] reported the positive correlation between CP and gas production at 24 h in Mediterranean browse species. Getachew *et al.*[18] reported that feed CP level was negatively correlated with gas production. However other studies with different types of feeds (i.e. CP ranging from 32 to 487 g kg^{-1} DM; Blümmel *et al.*[19]) have shown no effect of CP level on gas production.

The pattern of fermentation of feedstuffs was distinctly different, particularly at last times of incubation (Fig. 2). AP fermented faster and TP fermented slower than the other test feeds. AP had highest gas production volume among feedstuffs, the reason of more gas production volume in AP may be caused by present of high level of pectin and non structural carbohydrates. Regarding that NW had high level of starch and it fermented by microorganism to propionate, the gas production was lower in NW. The strong correlation between extent of gas production and chemical composition and the poor correlation between rate of gas production and chemical composition, is consistent with Nsahlai *et al.*[20].

Potential gas production was significantly different among feedstuffs (Table 5). AP had the highest potential gas production and TP had the lowest potential gas production. Metabolizable energy (ME), organic matter digestibility (OMD), short chain fatty acids (SCFA) and partitioning factor of the feedstuffs are shown in Table 5. The values for the ME, OMD and

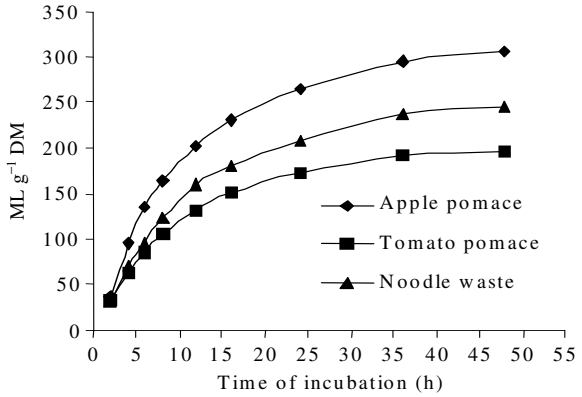

Fig. 2: Pattern of *in vitro* gas production on incubation of test feeds in buffered rumen fluid

Table 5: Gas production characteristics and estimated parameters

	Parameters					
Feeds	(a+b)[1]	c[2]	ME[3]	OMD[4]	SCFA[5]	PF[6]
Apple pomace	288.3[a]	0.084[b]	9.76	64.3	1.168	2.57
Tomato pomace	198.7[c]	0.09[a]	9.47	56.1	0.762	1.99
Noodle waste	265.6[b]	0.081[c]	8.87	57	0.919	3.47
SEM	0.0004	0.00006	-	-	-	-

1: Potential gas production (mL g^{-1} DM); 2: Rate constant of gas production during incubation (mL h^{-1}); 3: Metabolizable energy (MJ kg^{-1} DM); 4: OM digestibility (%DM); 5: Short chain fatty acid (mmoL); 6: Partitioning factor (mg degraded sample mL^{-1} gas production). The means within a column without common letter(s) differ ($p<0.05$)

SCFA ranged from in 8.87 NW to 9.76 in AP, 56.1 in TP to 64.3 in AP and 0.919 in NW to 1.168 in AP, respectively. Low determination of noodle waste's metabolizable energy can be resulted from its low rate of gas production and extent of gas production at 24 h. The high non-fiber carbohydrate content of noodle waste leads to proportionally higher propionate production, thereby reducing the acetate to propionate ratio[18]. Highly significant correlation has been observed between SCFA and gas production[21]. The molar proportions of different SCFA (acetate, propionate and butyrate) produced is dependent on the type of substrate[21]. Protein degradation leads to a proportionally smaller amount of SCFA. The extent of SCFA production from proteins is dependent upon on the amino acid composition of the feeds and the extent of rumen deamination of these amino acids. The carbon skeleton arising from deamination gives rise to a variety of VFA. For example, fermentation of glycine can lead to ammonia and acetic acid without the release of CO_2 and that of leucine, isoleucine and valine to isovaleric acid, 2-methyl butyric acid and isobutyric acids, respectively[18]. Partitioning factor in NW was highest and in TP was lowest, which indicated that in NW proportionally more substrate is converted into microbial biomass.

CONCLUSION

In the present study, feeds composition significantly affected the degradation parameters, when ruminal DM degradation of the various feed samples were considered. These results indicated that by-products can be used as replacement feedstuffs in diet for ruminants, but it needs to more research. It is concluded that *in situ* technique has a suitable correlation with gas production volume.

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