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EVALUATION OF RUMEN FERMENTATION KINETICS OF SOME BY-PRODUCTS USING *IN SITU* AND *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 brewers' grain, apple pomace, orange pulp and lemon pulp by-product using *in vitro* gas production technique. Test feeds had significant differences in chemical composition. Brewers' grain had the highest ($P<0.05$) crud protein (19.5 % DM) and ether extract (7.8 % DM). Apple pomace contained the highest neutral detergent fibre (43.3 % DM), acid detergent fibre (32.3 % DM) and lowest ash (3 % DM). Cumulative gas production was recorded at 2, 4, 6, 8, 12, 16, 24, 36, 48 and 72 h of incubation and the equation $Y = A\ (1-e^{-ct})$ was used to describe the kinetics of gas production. Potential gas production (A) and rates of gas production (c) differed ($P<0.05$) among feeds. Apple pomace showed higher potential gas production (A) (341 ml g^{-1} DM) and orange pulp had higher rate of gas production (c) (0.09 h^{-1}) than the other feeds. Inversely, lemon pulp (220 ml g^{-1} DM) had lower potential gas production than the other test feeds. The metabolizable energy (ME) (MJ kg^{-1} DM) content of feeds was calculated using gas production data. According to gas production data, the ME values ranged from 7.66 in lemon pulp to 10.83 MJ kg^{-1} DM in apple pomace. For *in situ* technique duplicate dacron bags were incubated for 0, 2, 4, 8, 12, 24, 48 and 72 h in two wethers fitted with ruminal cannulas. The model $y = a+b\ (1-e^{-ct})$ was used for determination of degradation characteristics. Orange pulp had higher soluble DM (a) (36.9 %), lemon pulp had higher insoluble potentially degradable DM (b) (62 %) and orange pulp had higher degradation rate (0.112 % h^{-1}) than other feeds. It was concluded that regarding different chemical composition of test feeds, the *in situ* dry matter degradability, *in vitro* gas production and ME, SCFA and OMD of feeds showed different values.

Key words: by-product; nutritive value; gas production; *in situ*; dry matter degradability

INTRODUCTION

A major constraint to increasing livestock productivity in developing countries is the lack 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 fulfil 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. Most by-product feedstuffs (BPF) result from the processing of commercial crops, the food processing industry and the fibre industry.

Tobias Marino *et al.* (2010) studied the potential of several vegetables and fruit wastes, that had expired the date of display in supermarket shelves, as a ruminant feed source by *in vitro* gas production technique. They reported that some vegetables and fruits have potential as a ruminant feed. However, low dry matter content of these feeds can interfere with the viability of their transport and utilization.

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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 (Durand *et al*., 1998; Sutton, 1986) and the knowledge about their potential feeding value is insufficient.

Considerable amount of grains are used in the brewery and distillery industry. The main by-products are distillers‘ grains and distillers‘ solubles. Approximately, 100 kg of grains provides ±33 kg distillers‘ dried grains with solubles or ±20 kg distillers‘ dried grains and ±32.5 kg condensed distillers‘ solubles with ±35 % DM (Mirzaei-Aghsaghali and Maheri-Sis, 2008). Apple pomace is the by-product of the production of cider and juice. It accounts for about 18.5 kg wet or 4.2 kg dried apple pomace per 100 kg apples. Dried apple pomace is a source of pectin (Boucque and Fiems, 1988). The extraction of the juice from citrus fruits provides citrus pulp as residue. Citrus pulp consists of 60-65 % peels, 30-35 % segment pulp and 0-10 % seeds.

There is little information available on the nutritive value of Brewers' Grain (BG), Apple Pomace (AP), Orange Pulp (OP) and Lemon Pulp (LP). The present study was, therefore, carried out to determine the chemical composition, digestibility and degradability of BG, AP, OP and LP.

There are several methods to evaluate feedstuffs. Determining the digestibility of feeds *in vivo* is laborious, expensive, requires large quantities of feed, and is largely unsuitable for single feedstuffs thereby making it unsuitable for routine feed evaluation. *In vitro* methods provide less expensive and more rapid alternatives (Getachew *et al*., 2004). Digestibility may be directly determined *in vivo* or estimated by using *in vitro* procedures, which are cheaper and more convenient (Aregheore, 2000). There are a number of *in vitro* techniques available to evaluate the nutritive value of feeds at relatively low cost such as *in vitro* gas production technique. The *in situ* nylon-bag technique is widely used to characterize the disappearance of feeds from the rumen (Woods *et al*., 2002). Nylon-bag technique provides a useful means to estimate rates of disappearance and potential degradability of feedstuffs and feed constituents (Getachew *et al*., 1998). Kamalak *et al.* (2005) compared *in vitro* gas production technique with *in situ* nylon bag technique to estimate dry matter degradation and reported that the *in vitro* gas production technique has good potential to predict *in situ* DM disappearance and some DM degradation parameters.

In this study, *in situ* and *in vitro* gas production techniques were used to describe nutritive value of by-products of the food industry for ruminants.

MATERIAL AND METHODS

Experimental feeds

The samples of BG, AP, OP and LP were collected from food industrial company in north-western Iran. BG and AP samples were moist and OP and LP samples were dry when collected. Samples of all test feeds for the *in situ* and gas production technique were milled through a 2.0 mm sieve and for chemical analyses they were milled through a 1.0 mm sieve. Moist samples were dried at 60°C before milling.

Chemical analysis

Samples of feeds and faeces were dried in an oven at 105°C for 24 h and the DM content calculated. Ground samples were analyzed for ash (AOAC, 2005). Determinations of N were conducted using the Kjeldahl method in an automated Kjelfoss apparatus (Foss Electric, Copenhagen, Denmark). Neutral-detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the detergent procedures of Van Soest *et al.* (1991). Ether extract (EE) was determined by extracting the sample with ether (AOAC, 2005).

In situ ruminal procedure

Two wethers fitted with rumen cannula were used to measure rumen degradability of feeds. The wethers were fed a diet composed of 385g.day^{-1} lucerne hay, 280g.day^{-1} barley grains, 35 g.day^{-1} wheat bran and 1.5 g.day^{-1} limestone at maintenance on DM basis (NRC, 1985). The wethers were kept in individual tie-stalls with individual feed bins in an animal house and had continuous access to water. Diets were given as total mixed ration with fresh feed offered twice a day (08:30 and 15:30 h). The nylon bag technique (Orskov and McDonald, 1979) was used to measure the DM degradation of feeds in the rumen. Nylon bags (4 cm×8 cm polyester bag; pore size 45-50 μm) containing 3 g of feed ground through a 2 mm screen were incubated in the rumen for 2, 4, 8, 12, 24, 48 and 72 h for feeds, immediately after the morning feeding. As a whole, there were four replicates for each feed sample and for each incubation time (2 wethers × 2 bags). Immediately after removal from the rumen, the bags were washed in cold water and frozen at −18°C. At the end of the collections, they were unfrozen and washed together with the zero time bags (not incubated in the rumen) for 20 min and then dried at 80°C for 24 h. The residues were weighed and submitted for analysis.

In vitro gas production

Samples (300 mg) were weighed into 100 ml serum vial. Mc Dougall (1948) buffer solution was prepared and placed in a water bath at 39°C. Rumen liquor samples were obtained from the two wethers used for *in situ* technique. Rumen fluid was collected after the morning feeding. Rumen fluid was pumped with a manually operated vacuum pump and transferred into pre-warmed thermos flask, combined, filtered through four layers of cheesecloth and flushed with CO_2. Each feed sample was incubated in five replicates with 20 ml of rumen liquor and buffer solution (1:2). Five vials containing only the rumen fluid/buffer solution and no feed sample was included with each test and the mean gas production value of these vials was termed the blank value. The vials were sealed immediately after loading and were affixed to a rotary shaker platform (lab-line instruments Inc Melors dark, USA) set at 120 rpm housed in an incubator. Gas production was measured in each vial after 2, 4, 8, 12, 16, 24, 36, 48 and 72 h of incubation using a water displacement apparatus (Fedorak and Hrudey, 1983).

Calculations and statistical analysis

In situ dry matter (DM), rapidly degradable fraction (a), potentially degradable fraction (b), and rate of degradation of fraction b (c), were calculated according the model of Ørskov and McDonald (1979) as $y = a+b\ (1-e^{-ct})$ that **y** is the actual degradation of DM after **t**, **a** is the intercept of the degradation curve at time zero, **b** is the potential degradability of the component of the insoluble but degradable DM, which will in time be degraded, **c** represents the constant of degradation rate of **b** at time t, **t** is incubation time.

Rate and extent of gas production was determined for each feed by fitting gas production data to the one component McDonald model: $Y = A\ (1- e^{-ct})$, where y is the volume of gas produced at time t, A the potential gas production (ml g^{-1} DM), and c the fractional rate of gas production. Parameters A and c were estimated by an iterative least square method using a non-linear regression procedure of the statistical analysis systems (SAS, 1999).

The metabolizable energy (MJ.kg^{-1} DM) content of feeds was calculated using equation of Getachew *et al.* (2002) as:

$$ME(MJ.kg^{-1}DM)=1.06+0.157GP+0.084CP+0.22CF-0.081CA$$

The short chain fatty acid (SCFA) and organic matter digestibility (OMD) for feeds were calculated using equations of Menke *et al.* (1979) as:

$$SCFA\ mmol.\ 200\ mg^{-1}\ DM = 0.0222\ GP - 0.00425$$

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

Where, GP is 24 h net gas production (ml/200 mg DM); CP, CF and CA are crude protein, crude fat and crude ash (% DM), respectively.

Data on *in situ* DM degradability and gas production parameters were subjected to one-way analysis of variance using the analysis of variation model (ANOVA) of SAS (1999). Multiple comparison tests used Duncan's multiple-range test (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Chemical composition

The chemical composition of test feeds is presented in the Table 1. The CP content of feeds ranged from 66g.kg^{-1} in LP to 195g.kg^{-1} in BG. The NDF content of feeds ranged from 124g.kg^{-1} in OP to 433g.kg^{-1} in AP. Apple pomace contained substantially higher OM level than the other feeds.

Variation in test feeds chemical composition has been observed compared to other studies with test feeds (Brabander, 1999, Kafilzadeh, 2008 and Bampidis and Robinson, 2006). The differences among chemical composition test feeds can be due to variation in varieties, cultivating and environmental conditions of feeds that are used in food industry. The feeds used in the current study were by-product feeds, which are created as a result

Table 1: Chemical composition of the test feeds (g.kg^{-1}). †

	Feedstuffs			
Variable	Brewers' grain	Apple pomace	Orange pulp	Lemon pulp
DM	368	311	849	872
CP	195	72	79	66
EE	78	29	18	32
NDF	219	433	224	209
ADF	129	323	153	164
Hemicellulose	90	110	71	45
Ash	49	30	47	51

† Three samples were analyzed for each feed

of processing of fruits for human foods, and therefore their composition varies depending on the composition of original plant material, method of processing, and type of components extracted or removed (Getachew *et al*., 2004).

***In vitro* gas production**

There was a difference (P<0.05) in gas production among feeds (Table 2). Potential gas production (A) and rates of gas production (c) differed (P<0.01) among feeds. The pattern of fermentation of test feeds was significantly different, particularly at first times of incubation (Figure 1). Apple pomace fermented faster and lemon pulp fermented slower than other test feeds.

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.* (1994). Low gas yield for lemon pulp in incubation times compared to the other test feeds resulted due to high content of ash and low content of crude protein. Low degradable CP was limiting microbial activity and low carbohydrate fractions degraded according to their potential. The high positive correlation among gas production, dry matter and organic matter digestibility was reported (Datt and Singh, 1995). Al-Masri (2003) reported a very highly significant (P<0.0001) relationship between gas production and the true and apparent fermented organic matter.

The high level of orange pulp gas yield in initial incubation times can be assumed that orange pulp had high level of soluble carbohydrate. Taghizdeh *et al.* (2008) reported that amount soluble carbohydrate can alter gas yield in initial incubation time.

Tamminga (1994) reported that particle size is one of the important factors that can alter gas yield and low particle size increase gas yield because microorganisms can be better attached and degradation rate can be increased. Citrus pulps have an especial physical structure

Table 2: *In vitro* gas production characteristics of feed samples incubated in buffered rumen fluid

	Gas production (ml. g^{-1} DM)										Gas production constants	
Feeds	2 h	4 h	6 h	8 h	12 h	16 h	24 h	36 h	48 h	72 h	A	c
BG	37^b	68^b	100^b	132^b	169^b	214^b	244^b	273^b	292^b	313^b	308^b	0.06^b
AP	40^b	85^a	122^a	158^a	209^a	236^a	279^a	302^a	315^a	364^a	341.3^a	0.07^b
OP	49^a	81^a	106^b	130^b	175^b	196^c	209^c	218^c	245^c	256^c	244.6^c	0.09^a
LP	39^b	66^b	81^c	98^c	141^c	166^d	183^d	202^c	219^d	220^d	220.9^d	0.07^b
SEM (n=5)	1.98	3.84	4.08	5.07	6.47	5.04	5.00	5.57	5.55	4.84	4.489	0.0001

a, b, c, d Means within a column with different superscripts differ (P<0.05).
c: fractional rate of gas production (h^{-1}); A: potential gas production (ml.g^{-1} DM).
BG = brewers' grain, AP = apple pomace, OP = orange pulp and LP = lemon pulp.

Table 3: The *in situ* disappearance of DM (%) and DM degradation characteristics of test feeds in the rumen

	DM degradability (% DM)								Degradation characteristics		
Feeds	0 h	2 h	4 h	8 h	12 h	24 h	48 h	72 h	a (%)	b (%)	C (%.h^{-1})
BG	21.7^d	27.9^d	32.2^c	41.5^d	51.4^d	58.0^d	66.0^c	69.8^c	21.6^d	47.0^c	0.070^b
AP	26.2^c	30.4^c	37.2^b	47.1^c	58.3^c	66.7^c	75.9^b	77.5^b	24.8^c	52.7^b	0.072^b
OP	40.1^a	42.7^a	54.6^a	68.8^a	75.9^a	85.4^b	87.4^a	87.6^a	36.9^a	51.3^b	0.112^a
LP	34.1^b	37.7^b	38.4^b	59.8^b	64.0^b	87.9^a	88.5^a	89.8^a	29.6^b	62.0^a	0.074^b
SEM (n=4)	0.473	0.576	0.700	0.722	0.621	0.552	0.613	0.667	0.541	0.834	0.0033

BG = Brewers' grain, AP = Apple Pomace, OP = Orange pulp and LP = Lemon Pulp.
a, b, c, d Means within a column with different subscripts differ (P<0.05).

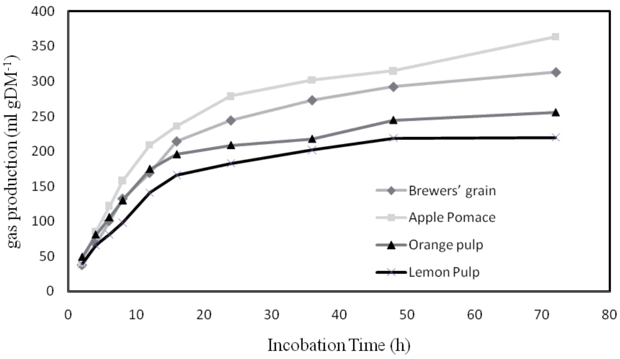

Fig. 1: Pattern of *in vitro* gas production on incubation of test feeds in buffered rumen fluid

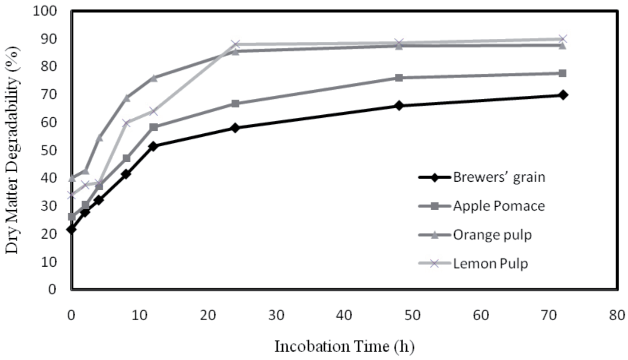

Fig. 2: Pattern of *in situ* degradability on incubation of test feeds in rumen

and are converted to powder when they are milled. In this study, citrus pulp had high gas yield in initial time and that's because of using low particle size (powder form) of the feeds during incubation.

Besharti *et al.* (2008) reported the value of 264 ml.g^{-1}DM for gas yield after 24h incubation for apple pomace and in this study the value was 279 ml.g^{-1}DM. This difference can result from variation in apple variety and apple pomace which can be a by-product of juice or puree making industry which they have different chemical and nutrient composition. Kafilzade *et al.* (2008) reported that apple pomace from puree making, had a higher soluble carbohydrate than apple pomace from juice.

***In situ* method**

Dry matter losses from the nylon bags incubated in the rumen and in situ DM degradability characteristics are presented in Table 3. There were differences ($P<0.05$) among test feeds in dry matter degradability after several incubation times. Total washing losses of DM (zero time bags) represented 21.7 to 40.1 % of DM in brewers' grain and orange pulp, respectively. Dry matter disappearance from nylon bags incubated in the rumen increased with increasing incubation time. The 72 h incubation time was sufficient for test feeds to be degraded.

A large range of dry matter degradation characteristics was obtained: the 'a', 'b' and 'c' values ranged from 21.6 to 36.9 % (for BG and OP), 47 to 62 % (for BG and LP) and 0.070 to 0.112 %h^{-1} (for BG and OP), respectively.

The relationship between the degradability parameters a, b and c and the chemical composition of 60 test feeds was reported by Woods *et al*. (2003). They reported that the slowly fermented structural carbohydrates are thought to play a dominant role in the degradation characteristics in the rumen. The high level of orange pulp and lemon pulp DM degradability in several incubation times can be assumed that small particle size of milled orange pulp and lemon pulp (because of especial physical structure) was not limiting microbial activity thus allowing the orange pulp and lemon pulp be degraded according to their potential.

In comparison to Pereira *et al*. (1998), DM degradation characteristics of brewers' grain - the 'a' and 'c' values were similar and 'b' value was different in this study. Where different degradability values are observed between this study and that of Pereira *et al*. (1998), it is possibly because of differences in nutrient composition, pore size of nylon bag and milling screen size.

Table 4: Evaluated SCFA, ME and OMD by *in vitro* gas production results

Feedstuffs	According to *in vitro* gas production data		
	SCFA mmol. 200mg^{-1} DM	ME MJ.kg^{-1} DM	OMD %
Brewers' grain	0.906	10.46	65.1
Apple pomace	1.236	10.83	70.7
Orange pulp	0.924	8.30	60.3
Lemon pulp	0.809	7.66	55.5

SCFA = short chain fatty acid, ME = metabolizable energy and OMD = organic matter digestibility

Metabolizable Energy (ME) and Short Chain Fatty Acids (SCFA)

According to studies Menke *et al*. (1979) and Getachew *et al.* (2002), SCFA, ME and OMD could be evaluated by 24 h *in vitro* gas production data. These results are shown in Table 4.

Low content of lemon pulp's SCFA, metabolizable energy and organic matter digestibility can result from its low rate of gas production, extent of gas production at 24 h and its nutrient composition.

CONCLUSION

In the present study, results indicated that by-products can be used as replacement feedstuffs in diets for ruminants. In an overall conclusion the nutritive value of apple pomace were better than other by-product feeds. However, all by-product feeds that were used in this study can be used economically as potential fibrous and energy sources in ruminant nutrition. As a whole, the wide variation in chemical composition of feedstuffs, gas production, rumen dry matter degradability, ME, SCFA and OMD offer users flexibility in formulating rations according to the productive performance of target animals.

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