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ORIGINAL ARTICLE

Use of Gas Production Technique to Evaluate Some Feedstuff in Ruminants

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ABSTRACT
In vitro gas production (GP) were used in order to study fermentation patterns of rice bran (RB), beet sugar pulp (BSP) and palm pulp (PP). The rumen fluid from ruminally-fistulated sheep were mixed with McDougall's buffer and added to fermentation vessels containing substrate, being RB, BSP and PP, which were evaluated in separate incubations at 39 ±0.5 ◦C for 96 h. Total GP was recorded after 2, 4, 6, 8, 12, 24, 48, 72, and 96 h of incubation. Metabolizable energy (ME), Net energy for lactation (NE_L), Microbial protein (MP), and rate of short chain fatty acids (SCFA) and digestibility of organic matter (DOM) were estimated at the end of the incubation. Also the approximate analysis of samples was determined. The resulted showed that, the PP was higher ether extract and lower ash and crude fiber than others. As well as, the rate of the produced gas was measured at all hours was related to PP. The highest and lowest amount of ME, NEL, MP, SCFA and DOM were observed for PP and BSP, respectively. This result indicated that, the nutritional value of PP was higher than RB and BSP in ruminants.
Keywords: *Beet sugar pulp, Gas production, Palm pulp, Rice bran.*

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INTRODUCTION

In vitro methods of feed evaluation have numerous advantages over *in vivo* methods. 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 [33], *in sacco* method of [22], and enzymatic method of [14] have all been widely used to predict digestibility of feeds, and used as a selection tool for screening feeds for nutritional quality. Studies [24] reported a strong correlation between metabolizable energy (ME) values measured *in vivo* and predicted from 24h *in vitro* gas production (GP) and chemical composition of feeds. The GP technique is a useful method to estimate rate and extent of feed degradation [4,5,8,13]. The GP provides more detailed information on fermentation kinetics of ruminant feeds than can be achieved by estimating disappearance of feed from fermentation media [10] and a number of different *in vitro* GP techniques have been developed. These techniques have been described in reviews by [31] and [12]. Use of glass syringes as vessels for incubating feeds with rumen fluid and monitoring accumulated gas [23,24] is a well-known *in vitro* GP method. This method has also been widely used to evaluate the energy value of several classes of feeds [12], particularly straws [18,19], agro-industrial by-products [16], compound feeds [1] and various tropical feeds [17]. The technique has also been used to assess effects of anti-nutritive factors on rumen fermentation of feeds [7,15,26]. Therefore, the objective of this study was to use the *in vitro* GP technique to evaluate the ME, NE_L, DOM, MP and SCFA values of RB, BSP and PP that produced in native condition of Iran.

MATERIALS AND METHODS

Samples analysis

Samples of PP, RB and BSP were ground to pass a 1mm sieve and distributed to the participating laboratories. Test feeds used in the experiment was analyzed for DM by drying at 102^{o}C for 16 h in a forced air oven, and for CP, crude fat, crude fiber and ash (Table 1) according to methods 976.06, 920.39, 978.10 and 942.05, respectively, of AOAC [2].

Rumen fluid collection

Rumen fluid was collected from three rumen-fistulated sheep (6 years of age; 50±2.0 kg) about three hours after the morning feeding, transferred to the laboratory in a water bath preheated to 39±0.5 ^{0}C, squeezed through four layers of gauze, combined among sheep and purged with CO2. Sheep were housed separately in pens and fed a diet consisting of 700 g/kg meadow hay and 300 g/kg barley grain with free access to water.

Incubations and GP

Incubations were completed using 30ml of McDougall's [20] buffered rumen fluid according to [24]. Approximately 200mg of feed was weighed and placed into a 100ml graduated glass syringe. Pistons were lubricated with Vaseline and inserted into the syringes. Buffer and mineral solution was prepared and placed in a water bath at 39^{0}C under continuous flushing with CO_2. Rumen fluid (i.e. liquid and fine particles) was collected from animals into a pre-warmed thermos flask, and then filtered and flushed with CO_2. The mixed and CO_2-flushed rumen fluid was added to the buffered mineral solution (1:2 (v/v)), which was maintained in a water bath at 39^{0}C, and combined. Buffered rumen fluid (30ml) was pipetted into each syringe containing feed samples and the syringes were immediately placed into an incubator with a rotating disc, as described in some research [24], or into water bath at 39^{0}C [5]. Three syringes with only buffered rumen fluid were incubated and considered as the blank incubation. Each incubation was completed in triplicate per feed samples. Where a water bath was used, syringes were shaken gently every 2h, and GP was recorded after 2, 4, 6, 8, 12, 24, 48, 72, and 96 h of incubation. Total gas values were corrected for blank incubation.

Calculations

The reported 24h gas values were expressed per 200mg of DM. The metabolizable energy (MJ/kg DM), NE_L (MJ/kg DM), DOM (% DM) and SCFA (m mol/200 mg DM) content of feeds was calculated using equations of [23, 24]. As well as, SCFA was calculated by method of [11] and microbial protein (MP, g/kg DM) estimated as 19.3 g microbial nitrogen per kg DOM [9].

ME (MJ/kg DM) = 1.06 + 0.157GP + 0.084CP + 0.22CF − 0.081CA

NEL (MJ/kg DM) = -0.36 + 0.1149 GP + 0.0054 CP + 0.0139 CF – 0.0054 CA

DOM (% DM) = 9.00 + 0.9991 GP + 0.0595 CP + 0.0181 CA

SCFA (m mol/200 mg DM) = 0.0222 GP – 0.00425

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

Statistical analysis

Data from gas production, ME, NEL, DOM, MP and SCFA predicted from gas production at 24 h and chemical components were analyzed using the general linear models of SAS (SAS Institute Inc., Cary, NC, USA). Statistical significance of differences among treatments was assessed using the Duncan's test [30].

RESULTS

Chemical composition of test feeds showed that, the PP was higher ether extract and lower ash and crude fiber than RB and BSP (P<0.05; Table 1). This differences were reflected in rate and volume of GP of feeds (P<0.05; Table 2). In all time of incubation, the GP of PP was higher than others. The gas volume of test feeds ranked from the highest to the lowest was PP, RB and BSP (Fig. 1). As well as, the ME, NEL, DOM, SCFA and MP of PP were higher than others (Table 3).

There are many factors affecting chemical composition of feedstuffs such as stage of growth, maturity, species or variety [35,27], drying method, growth environment [25] and soil types [32]. These factors interact to confer an influence on the nutritive value obtained for the agricultural wastes in this study. In addition, low crude fiber and ash result in higher GP of PP than others. As well as, high ME, NEL, DOM, MP and SCFA of PP due to its low ash and high CP and EE content.

For gas volume and *in vitro* GP characteristics, researchers suggested that gas volume at 24h after incubation is an indirect relationship with metabolisable energy in feedstuffs [23]. The GP can be regarded as an indicator of carbohydrates degradation, [28,29] suggested 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. The GP is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate. As well as, GP from protein fermentation is relatively small as compared to carbohydrate fermentation while contribution of fat to GP is negligible [36]. The fastest rate of GP was

observed in PP, possibly influenced by the soluble carbohydrate fraction readily available to the microbial population. Slower rates were observed in RB and BSP indicating that these residues were less readily available to the microbes in the rumen (Fig. 1).
Although GP is a nutritionally wasteful products but provides useful basis from which ME, NEL, DOM, MP and SCFA may be predicted [3]. There was a positive correlation between metabolisable energy calculated from *in vitro* GP together with CP and fat content with metabolisable energy value of conventional feeds measured *in vivo*, [24]. Using the *in vitro* gas measurement and chemical composition in multiple regression equation [21,23,28,34] found a high precision in prediction of *in vivo* DOM.
High ME, NEL and DOM in PP was due to high GP and crude fat and low ash content in this by-product than others. The MP synthesis was estimated from DOM (19.3 g MP/ kg DOM; 9]. Thus such as DOM, highest and lowest MP were observed in PP and BSP, respectively (Table 3). In addition, there were significant differences in SCFA among the feedstuffs, with lowest for BSP. The least SCFA estimated for BSP is due to a lower GP which was evident in the first 24h of incubation (Fig. 1). Some studies [6] suggested that GP from cereal straws and in different classes of feeds incubated in vitro in buffered rumen fluid was closely related to the production of SCFA which was closely related to the production of SCFA which was based on carbohydrate fermentation. Since PP had the highest estimated SCFA compared with other agricultural wastes, suggests a potential to make energy available to the ruminants. Other research [11] reported a close association between SCFA and GP.

Table 1. Chemical composition of the test feeds (% DM)

Feedstuff	DM	CP	EE	CF	Ash
Palm pulp	96.15	7.36 ^{c}	6.5 ^{a}	22.8 ^{c}	1.81 ^{c}
Rice bran	95.77	6.54 ^{b}	3.7 ^{b}	34.4 ^{a}	16.21 ^{a}
Beet sugar pulp	96.27	9.16 ^{a}	1.3 ^{c}	32.4 ^{b}	15.26 ^{b}
P	0.0505	<0.0001	<0.0001	<0.0001	<0.0001

Assays represent duplicate sample per feedstuff. DM: dry matter; CP: crude protein; EE: ether extract; CF: crude fiber

Table 2: *In vitro* GP (ml/200 mg DM) of the test feeds

Treatment	V2	V4	V6	V8	V12	V24	V48	V72	V96
PP	9.17 ^{a}	19.43 ^{a}	22.57 ^{a}	26.33 ^{a}	31.03 ^{a}	45.77 ^{a}	68.97 ^{a}	74.93 ^{a}	78.22 ^{a}
RB	4.83 ^{b}	10.45 ^{b}	12.17 ^{b}	14.88 ^{b}	19.19 ^{b}	23.71 ^{b}	28.09 ^{b}	30.59 ^{b}	31.98 ^{b}
BSP	5.17 ^{b}	11.12 ^{b}	12.53 ^{b}	14.88 ^{b}	17.55 ^{b}	20.70 ^{c}	25.39 ^{c}	26.96 ^{c}	29.15 ^{c}
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^{a,b,c} Means within the same column with different superscripts are significantly different, P < 0.05.
PP: palm pulp, RB: rice bran; BSP: Beet sugar pulp

Table 3: Estimation of digestive parameter of test feeds

Treatments	ME (MJ/kg DM)	NEL (MJ/kg DM)	DOM (% DM)	MP (g/kg DM)	SCFA (mmol/200mgDM)
PP	10.07 ^{a}	5.01 ^{a}	55.15 ^{a}	66.52 ^{a}	1.01 ^{a}
RB	4.91 ^{b}	2.37 ^{b}	33.42 ^{b}	40.32 ^{b}	0.52 ^{b}
BSP	4.13 ^{c}	2.33 ^{c}	30.49 ^{c}	36.78 ^{c}	0.45 ^{c}
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^{a,b,c} Means within the same column with different superscripts are significantly different, P < 0.05.
PP: palm pulp, RB: rice bran; BSP: Beet sugar pulp

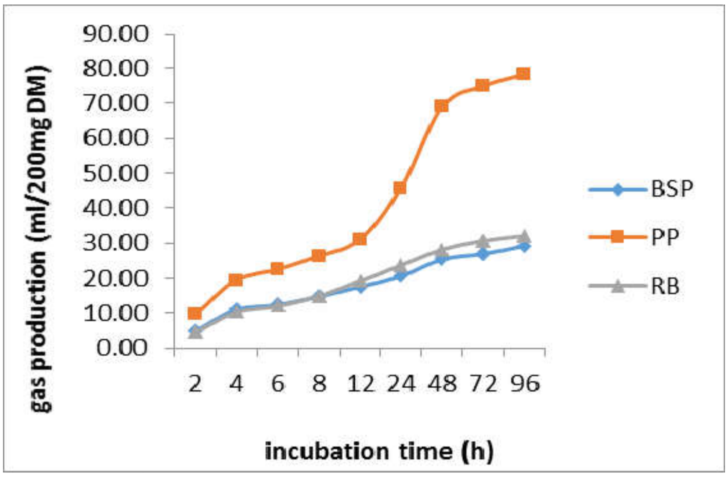

Figure 1- Relationship between test feeds and gas production

CONCLUSION

The *in vitro* GP techniques can be used to assess the nutritive value of tropical agricultural wastes and to differentiate between their potential digestibility and metabolisable energy contents. Chemical composition and *in vitro* digestibility are very useful in estimation of DOM, SCFA, NEL and ME. This result indicated that, the nutritional value of PP was higher than RB and BSP and revealed that this agricultural by-products possess the potentials of being included in the diet of ruminant.

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