Estimation of organic matter digestibility and metabolizable energy content of agro-industrial wastes using *in vitro* gas production

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Abstract

Organic matter (OM), neutral detergent fibre (NDF) digestibilities and metabolizable energy content of some available agro-industrial wastes (AIW) such as dried brewers' grains, cocoa shell, cocoa dust, desiccated coconut waste meal, wheat bran and rice bran used as ruminant feeds were estimated from *in vitro* gas production technique. Their crude protein (CP) content varied from 13.7 – 25.6 %, NDF ranged from 21.9 to 48.4 %, OM from 88.6 – 95.4 % and non-structural carbohydrate (NSC) estimates increased from 7.2 – 45.6 %. Desiccated coconut waste meal (DCWM) had the lowest NSC content. Nutrient contents varied among the AIW. Net gas production ranged from 7.7 – 23.1 ml/24 h and significant differences ($P < 0.05$) were observed among the AIW in net gas production. The lowest and highest gas productions were obtained in cocoa dust (CD) and wheat bran (WB), respectively. True dry matter and NDF digestibility were also significantly different ($P < 0.05$) among the AIW. True dry matter and NDF digestibility ranged from 61.1 – 76.0 and 52.4 – 64.1 %, respectively. OM digestibility and ME (MJ/kg of DM) values showed significant differences among the AIW. AIW with high OM digestibility also had higher ME values. Statistical significant differences ($P < 0.05$) were obtained among the AIW in the rate c (ml/h⁻¹) and potential extent b (ml) of gas production. Chemical analysis data proved that they possess reasonable CP, varied NDF, OM and gross energy (MJ/kg DM). *In vitro* digestibility data demonstrated that net gas production NDF and OM digestibility and ME (MJ/kg DM) varied based on the type of AIW. The results obtained demonstrated that from chemical composition and net gas production, estimates of nutrients digestibility (OM, NDF and true dry matter) of AIW in the Pacific Island countries could be readily accomplished by the *in vitro* gas production techniques. The AIW investigated had OM digestibility of between 17.9 – 47.6 % and ME values that ranged from 4.2 – 6.5 MJ/kg DM. Finally this investigation demonstrated that available AIW in the Pacific Island countries have potential as animal feedstuffs.

Keywords: Agro-industrial waste, *in vitro*, organic matter, neutral detergent fibre, digestibility, metabolizable energy, gas production

Introduction

Agro-industrial wastes (AIW) may be defined as those materials that remain after the main product of interest has been removed or extracted in an industry. They result from the processing of crops and in most situations confined to factory sites. Some could be marketed and exported for foreign exchange. These products are now being evaluated to determine their suitability as animal feeds in the small Pacific Island countries in the South Pacific region. Agro-industrial wastes of economic value in the region are brewers' grains, cocoa by-products (cocoa shell and cocoa dust), desiccated coconut waste, wheal bran and rice bran. Most of these by-products are discarded in the small Pacific Island countries.

Brewers' grains, the most important by-product resulting from the manufacture of beer, are the extracted residues of malt (generally barley). They contain the insoluble material remaining after the process of mashing and cooking with water which include the fibre fractions, fats, proteins, together with residues of starch and dextrin. It constitutes an important by-product and the major use of this material has been as a feed for livestock. After drying that is performed to allow storage and improve nutrient concentration, the product becomes known as dried brewers' grains. The concentration of fibre fractions and the low protein degradability means that brewers' grains are preferentially used for feeding ruminants (Bovolenta *et al.* 1998). In most Pacific Island countries, it is available and wasted. The product can be fed wet or dry to livestock.

Cocoa shell (CS) and cocoa dust (CD) are the waste by-products from the manufacture of chocolate. There is dearth of information on the utilization of cocoa shell (CS) and cocoa dust (CD) in ruminant nutrition in the region, however,

their utilization in livestock rations have been reported (Hutagalung, 1978; Greenwood-Barton, 1985). The value of these byproducts as possible livestock feed has received very little attention in the Pacific Island countries because they have not been previously considered important in ruminant nutrition (Aregheore, 2001a).

Desiccated coconut waste meal (DCWM) is one of two important by-products of the coconut industry. DCWM is a by-product from coconut cream production and has tremendous potential as a feed resource in the region. DCWM is often discarded in both urban and rural areas. As a feed it is ideal for ruminants either in the wet or dry form, however, in the wet form it does not keep for a long time before fermentation sets in (Aregheore and Tunabuna, 2001).

Wheat bran or mill mix is a by-product of flourmills and it has a wide acceptance in the diets of livestock. This is commercially available and extensively used in ruminant nutrition where it exists. Wheat bran could be low or high in fibre depending on the processing methods used and it is usually low cost and low energy material. It is used by feed-millers when they want to make cheap or formulate low energy diets (Olomu, 1996).

Rice bran is the ungraded by-product obtained in the processing of rice grains for human consumption. It is a good concentrated ingredient for ruminants. It has a moderate concentration of fibre and it is used as filler in most formulated diets for livestock (Aregheore, 2001b).

These AIW could contribute to diets of ruminant livestock in the region if properly harnessed and processed. They contain little economical value as edible foods for human consumption therefore they could be used as major sources of dietary

nutrients and energy in livestock production. They could account for the entire non-forage portion of the diet of ruminant livestock in the Pacific Island countries.

In the Pacific Island countries, little research has been done to characterize them for nutrient contents and evaluate them as potential feed resources. However, it has been observed that some significant variability exist in chemical composition of some available AIW (DePeters, *et.al.* 1997) At present attempts are therefore been made to include them in rations for ruminants (Aregheore, 2000, 2001b, 2001c, 2002).

Cumulative gas production from the incubation of feedstuffs using rumen liquor or fresh faecal samples suspension as inoculum *in vitro* together with crude nutrient composition can be used to predict organic matter digestibility and metabolizable energy content of ruminant feeds with high accuracy (Menke *et al.* 1979; Menke and Steingass, 1988; Akher *et al.* 1999). There is scant information on the use of gas production techniques to determine OMD and ME of AIW. The objectives of this study were to estimate the organic matter digestibility and metabolizable energy content of some agro-industrial wastes from the Pacific Island countries used as ruminant feeds from *in vitro* gas production technique.

Materials and Methods

Feed ingredients preparation.

Brewers' grains were collected wet from Western Samoa Breweries Ltd, Apia. These were spread in an open but concrete floor, turned regularly until they were dry. Desiccated coconut waste meal was collected wet from Samoa Tropical Products LTD, Apia. This was dried under shade for 4 - 7 days until the product became brown.

During the period the product was continuously turned for even drying and proper aeration. Cocoa shell (CS) and cocoa dust (CD) were obtained from Wilex C & C Products Ltd., Moatia, Apia. Wheat bran and rice bran were obtained from the Flourmills of Fiji (FMF), Suva and Rewa Rice Mill, Nasourii, Fiji, respectively. ***Nutritional evaluation using in vitro gas method***

Samples were incubated *in vitro* with rumen fluid in calibrated glass syringes following the procedure of Menke and Steingass (1988). Rumen liquor was obtained from three mature sheep (crossbred Suffolk; mean live-weight of 65 kg) fed with 800g DM timothy hay (*Phleum pratense*) and 200g DM concentrate, twice daily and had free access to water and mineral mix. About 200 mg of 1 mm milled samples were weighed into 100 ml calibrated glass syringes in duplicate. Pure oil was applied to the piston to ease movement and to prevent escape of gas.

The syringes were pre-warmed (39° C) for 1 h, before addition of 30±1.0 ml of rumen-buffer mixture into each syringe. All the syringes were incubated in a water bath maintained at 39±0.1°C. Incubations were stopped after 24 h incubation and gas volume (Gv) was noted. Organic matter digestibility (OMD, %) and metabolizable energy (ME, MJ kg⁻¹ DM) were calculated using the following equations (Menke and steingass, 1988):

OMD = 14.88 + 0.889* Gv + 0.45* CP + 0.65CA,
and ME = 2.20 + 0.136* Gv + 0.057* CP + 0.0029CF
Where Gv is 24 h. net gas production (ml/200 mg DM), CP, CA, and CF are crude protein, crude ash and crude fibre (%DM), respectively. These equations have been standardized and validated as method to create the OMD and ME prediction equations using data from 400 digestibility trials (in vivo) and the corresponding in vitro gas production tests (Menke and Steingass, 1988).

True dry matter digestibility

500 mg of samples were incubated in a buffered medium containing rumen liquor (40 ml). True dry matter digestibility was determined after 24 h of incubation treating the syringe content with neutral detergent solution to obtain NDF. The determination of true dry matter (DM) and neutral detergent fibre (NDF) digestibility were according to the procedures of Van Soest and Robertson (1985);

$$\% \text{ True dry matter digestibility} = \frac{(\text{Truly digested substrate (DM)}) \times 100}{(\text{Weight of sample DM taken for incubation})}$$

Rate and potential extent of gas production

The samples (200-mg) were incubated in triplicate in graduated syringes containing 30 ml of *in vitro* medium containing rumen liquor (Menke *et al.* 1979). At 2, 4, 6, 8, 10, 12, 24, 36, 48, 54, 60, 72 and 96 h, gas values were recorded. The mean gas volume readings were fitted to the exponential equation $p = a + b(1 - e^{-ct})$ (Ørskov and McDonald, 1979). Where p = gas production at time t ; $a + b$ = the potential gas production, c = the rate of gas production and a , b , and c are constants in the exponential equation using Neway' computer program (X.B.Chen, Rowett Research Institute, Aberdeen, Scotland).

Proximate analysis

Proximate components were analyzed according to AOAC (1995). Fibre analyses [neutral detergent fibre (NDF) and acid detergent fibre (ADF)] were determined by the procedures of Van Soest *et al.* (1991).

Statistical analysis

Data obtained were analyzed by ANOVA and significant differences between means were compared using Duncan's multiple range tests with the aid of SAS/STAT program (Statistical Analysis Systems Institute Inc., 1988).

Results and Discussion

Table 1 presents the chemical composition of the agro-industrial wastes (AIW). The chemical composition of the AIW varied. Crude protein (CP) content varied from 13.7 in RB to 25.6 % in DBG, while NDF contents ranged from 21.9 % in RB to 43.2 % in CS. Organic matter (OM) content was in the range of 88.6 – 95.4 %. There were also marked variations in the ether extract (EE) contents of the AIW (5.5 – 27.7 %). DCWM had the highest EE content followed by CD. The high EE value for DCWM and CD could pose problems such as rumen acidosis and/or displaced abomasum if they are often used as source of fat or energy in the nutrition of livestock in the Pacific Island countries. DePeters *et al.* (1997) reported that the inclusion of by-products high in EE could pose problems if they are often used as sources of fat in ruminant diets.

Proximate chemical composition values reported for these AIW are within the values reported by Devendra and Gohl (1970); Gohl, (1981); Oyenuga, (1968) and Aregheore (1992) for similar by-products. The non-structural carbohydrate (NSC) content of the AIW was calculated using the formula $NSC = [100 - (\text{Ash} + \text{CP} + \text{EE} + \text{NDF})]$. The NSC estimates for the AIW increased from 7.2 – 45.6 %. DCWM had the lowest NSC content. It has been observed that in most situations the NSC estimates of AIW are under estimated and implications of such under estimation with respect to production performance of ruminants are unknown (DePeters *et al.* 1997).

Net gas production, true dry matter (TDM), neutral detergent fibre (NDF) and organic matter (OM) digestibilities and ME (MJ/kg of DM) are presented in table 2. Net gas production ranged from 7.7 – 23.1 ml/24 h. and significant differences ($P < 0.05$) were observed among the AIW in net gas production. The lowest and highest gas

Table 1: Chemical composition of the Agro-Industrial wastes

Nutrients (%)	Agro-Industrial wastes					
	DBG	CS	CD	DCWM	WB	RB
Dry matter	89.5	93.0	88.0	89.6	90.4	92.0
Crude protein	25.6	13.8	15.9	22.2	19.6	13.7
Ether extract	5.5	11.0	22.0	27.7	6.7	15.3
Neutral detergent fibre	41.4	43.2	38.3	42.1	40.8	21.9
Acid detergent fibre	26.6	29.4	24.0	28.6	11.4	16.1
Organic matter	95.4	91.0	94.0	95.2	88.6	93.3
Non-structural carbohydrates	26.9	23.0	16.8	7.2	45.6	44.4
Gross energy (MJ/kg DM)	21.6	21.3	23.3	25.1	12.9	18.6

DBG - Dried brewers' grains; CS - Cocoa shell; CD - Cocoa dust; DCWM - Desiccated coconut waste meal; WB - wheat bran; RB - Rice bran.

Table 2: True dry matter, neutral detergent fibre (NDF %) and organic matter digestibilities and metabolizable energy (ME, MJ/kg of DM) Agro-Industrial wastes*

Parameters	DBG	CS	CD	DCWM	WB	RB	SEM
True dry matter digestibility	74.7a	61.1b	60.1b	71.8a	76.0a	63.1b	6.55
NDF digestibility	64.1a	61.5a	60.4a	62.8a	63.2a	52.4b	3.91
OM digestibility	47.6a	36.8b	17.9c	42.5a	44.1a	34.3b	9.94
ME (MJ/kg of DM)	6.4	4.2	4.8	5.3	6.5	4.5	0.89

*DBG - Dried brewers' grains; CS - Cocoa shell; CD - Cocoa dust; DCWM - Desiccated coconut waste meal; WB - wheat bran; RB - Rice bran.

^{a,b,c} Mean with different letter within rows are significantly different ($P < 0.05$)

SEM - Standard Error of Mean

production was obtained in cocoa dust (CD) and wheat bran (WB), respectively. Gas production generally reflect the contents of fermentable carbohydrate or carbohydrate degradation and also probably the nitrogen and lipids (Menke and Steingass, 1988; Blummel *et al.* 1997; Steingass *et al.* 1997).

True dry matter and NDF digestibilities were also significantly different ($P < 0.05$) among the AIW.

True dry matter and NDF digestibilities ranged from 61.1 – 76.0 and 52.4 – 64.1 %, respectively. The values obtained for the AIW in NDF digestibility are close to values reported for some agro-industrial byproducts by DePeters *et al.* (1997). True dry matter digestibility values obtained seems to portray the probably extent of NDF digestibility of the AIW. However, there were no value differences between true dry matter and NDF digestibilities in CS and CD (Table 2).

Organic matter (OM) digestibility showed significant differences ($P<0.05$) among the AIW. However, among the AIW, cocoa dust was an exception because it had a comparably low OM digestibility. The low OM digestibility obtained for the CD may not be unconnected with the presence of the alkaloid, *theobromine* it contained (Aregheore, 2001b). The presence of this anti-nutritive factor could affect the bioavailability of nutrients therefore making it impossible for microbes to utilize CD for the production of useful volatile fatty acids the major sources of energy to the ruminant livestock.

The rate c (ml/h^{-1}) and potential extent b (ml) of gas production of the AIW are presented in Table 3 and statistical significant differences ($P<0.05$) were obtained among the AIW. A comparison of gas production amongst the AIW and concomitant *in vitro* OM and NDF digestibilities indicated that gas production reflects actually substrate fermentation. The potential extent of gas production followed the trend of OMD (Table 3).

The utilization of roughages is largely dependent on microbial degradation therefore the rate and potential extent of gas production would provide a useful basis for the evaluation of the AIW as potential feed resources. Since gas production is dependent on the relative proportion of soluble, an insoluble but degradable and undegradable particles of feed, mathematical description of gas production profiles allows evaluation of substrate and fermentability of soluble and slowly fermentable components of feeds (Getachew *et al.* 1998).

Based on the above assumption, therefore, it could be adduced that among the AIW studied, CS and CD would provide minimal proportion of residue that would take up space if utilized in *in vivo* studies and also persists as indigestible residues. Ørskov and Ryle (1990) reported that the rate (c) determines digestion time and consequently how long a potentially digestible material would occupy space. Therefore the potential extent of digestion (b) values obtained for DBG, DCWM and WB demonstrated that

Table 3: The rate (c) and the potential extent (b) of gas production of the Agro-Industrial wastes

Agro-industrial wastes	Gas Production		
	Net gas produced (ml/24 h)	Rate c (ml/h^{-1})	Potential b (ml)
Dried brewers' grains	20.5a	0.0276b	44.696a
Cocoa shell	7.7c	0.1979a	8.504c
Cocoa dust	13.6b	0.2195a	10.239c
Desiccated coconut waste meal	8.9bc	0.0761b	17.669b
Wheat bran	23.1a	0.1046a	19.436b
Rice bran	11.5b	0.0701b	9.667c
SEM	5.73	0.069	12.48

^{a,b,c} Mean with different letter within a column are significantly different ($P<0.05$)

SEM – Standard Error of Mean

they possess more potentially degradable and fermentable carbohydrates than CS, CD and RB. Also the results presented in Table 3 actually demonstrated that digestion rate (c) and potential extent (b) of gas production provided a more meaningful index of nutritional value than ultimate digestibility comparatively. However, the conversion of true fermented organic matter into gas varied with the type of AIW.

Conclusion

In conclusion the AIW investigated possessed reasonable CP, varied NDF and OM and gross energy (MJ/kg DM). *In vitro* digestibility data demonstrated that net gas production, NDF and OM digestibilities and ME (MJ/kg DM) varied based on the type of AIW. The results obtained demonstrated that from the chemical composition and net gas production, estimates of nutrients digestibility (OM, NDF and true dry matter) of AIW in the Pacific Island countries could be readily accomplished by the *in vitro* gas production techniques. The agro-industrial wastes investigated had OM digestibility of between 17.9 – 47.6 % and ME values that ranged from 4.2 – 6.5 MJ/kg DM. Finally this investigation demonstrated that available AIW in the Pacific Island countries have potential as ruminant livestock feedstuffs if properly harnessed.

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