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Evaluation of degradability of Moringa, Pineapple waste and Plantain herbs by *in situ* and *in vitro* gas production technique

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ABSTRACT

Nutritional evaluation of feed ingredients is important for potential use in feed rations. It is very important to determine not only the nutrient content and quality of feeds but also their digestibility prior to adding in the ration. This study was undertaken to assess the potential nutritive value of selected shade-dried herbs of moringa, pineapple waste, and plantain through *in situ* degradation and *in vitro* gas production techniques. The study compared 4 treatments, namely moringa (MH), pineapple waste (PwH), plantain (PH) herbs, and a combination of three of them with a ratio of 1:1:1 (MxH). The rumen dry matter (DM) and crude protein (CP) degradability kinetics were analyzed by using the nylon bags (in situ) procedure and the organic matter digestibility and fermentation characteristics were analyzed by *in vitro* gas production technique. The results of the *in situ* study revealed that the ruminal dry matter disappearance differed significantly ($P<0.001$) among herbs at all incubation periods whereas the PH had the greatest disappearance and PwH had the lowest among all herbs. Significantly ($P<0.05$) the highest potential and effective degradability of dry matter were found in PH and the lowest in PwH among herbs. The ruminal CP disappearance differed significantly ($p<0.05$) among herbs at all incubation periods. The MH had the greatest CP disappearance whereas PwH had the lowest. Significantly ($P<0.05$) the highest potential and effective degradability of CP were found in MH and MxH, respectively, and the lowest in PwH among herbs. The results of the *in vitro* study revealed that cumulative gas production was increased with increasing the periods of incubation for all herbs. Significantly ($P<0.05$) the highest gas production was found in PH and MH among all herbs at 48h of incubation. The organic matter digestibility and metabolizable energy were found significantly ($P<0.05$) higher in MH & PH and lower in PwH. Short-chain fatty acids were also significantly ($P<0.05$) higher in MH & PH and lower in PwH among herbs. It may be concluded that both plantain and moringa herbs showed better potential and effective degradability and also higher organic matter digestibility and metabolizable energy comparable to pineapple waste herbs.

Introduction

The demand for animal-origin protein has increased due to the rapidly growing population. Domestic ruminants are the major source to supply the growing demand for animal-origin protein (Badhan et al., 2018). Agricultural land is steadily shrinking as the global population rises (Karakuş et al., 2019). Therefore, it requires increasing animal productivity by introducing new feedstuffs and enhancing the nutritional value of the existing feedstuffs (van der Poel et al., 2020). Nutritionists have been interested in nutritional tactics to boost ruminant output for several years through utilizing feed additives (Wenk, 2000) such as probiotics, prebiotics, enzymes, organic acids, and phytobiotics. In the past few decades, antibiotic in animal nutrition

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has been practiced as feed additives for the purposes of rapid growth, increasing feed efficiency, and prophylaxis (Cully, 2014 and Rushton, 2015). The harmful effect of multidrug-resistant bacteria has raised questions about the use of antibiotics as feed additives in animal production. According to previous studies, several plant species and their constituent parts may be used as alternatives to antibiotic substitutes for controlling rumen fermentation, lowering ruminal methane production, and enhancing animal performance (Lin et al., 2012). Medicinal herbs contain bioactive compounds, essential oils, saponins, flavonoids, tannins, and polyphenols that have been concentrated in their anti-microbial activity (Bodas et al., 2012), to decrease rumen methane emissions (Patra et al., 2006) or to modify the lipolysis and biohydrogenation of polyunsaturated fatty acids (Vasta et al., 2009; Jayanegara et al., 2011). Nutritional evaluation of feed ingredients for potential use in feed ration is key. These include not only assessing the nutrient content and quality of ingredients but also assessing their digestibility (Ayasan et al., 2020) or degradability coefficients (McDonald et al., 2010). Numerous techniques have been developed to assess the digestibility of feeds, including *in vivo*, *in situ*, and *in vitro* digestibility. The i*n vivo* feed evaluation in ruminants is not suitable for routinely used methods because it is time-consuming, expensive, requires huge amounts of feed and is mostly inappropriate for single feedstuffs. *In situ* and *in vitro* methods are commonly used to evaluate the rumen degradability or the percentage of feedstuff that is degraded in the rumen. The *in situ* nylon bag procedure used the degradability of feedstuffs in a nylon bag and incubated in the rumen of a cannulated animal and the disappearance of the sample at any one point can be predicted mathematically (Mehrez & Ørskov, 1977). Another method is gas production through *in vitro* technique that makes use of the principles of gas production from the fermentation of feeds by microorganisms in the test tube (Menke & Steingass, 1988). Even though these methods are quicker and more accurate, they need an inoculum to generate a fermentative environment (Mould et al., 2005) since they use less substrate than *in situ* techniques. However, there is limited published information regarding the ruminal degradability and *in vitro* digestibility of selected shade-dried herbs such as moringa, pineapple waste, and plantain. In this context, it is necessary to evaluate the nutritional value of selected medicinal herbs and verify the possibility of using their mixture at appropriate levels to formulate rations to improve animal performance and quality products. Thus, the study was undertaken to assess the potential nutritive value of selected shade-dried herbs through *in situ* degradation and *in vitro* gas production techniques.

Materials and Methods

Collection and preparation of herbs

Herb samples of moringa (*Moringa oleifera*) leaves, twigs, and branches were collected from the previously established moringa research plots of Bangladesh Livestock Research Institute, Savar, Dhaka. Herb samples of pineapple waste (*Ananas comosus*) (crown, peel, and leaf), and plantain (leaves) (*Plantago lanceolata*) were collected from the herbs bank of Shahjalal Animal Nutrition Field at Bangladesh Agricultural University, Mymensingh. Dirt and other unwanted particles were removed from the collected herbs sample and then cut manually into 3-4 cm in size. The collected samples were shade-dried under natural airflow. After drying, the herbs samples were ground in an electric grinder to pass through a 1-mm sieve and packed in an air-tight plastic sample container and stored in a freeze at -20^{0}C for chemical analysis *in situ* and *in vitro* experimental trial.

In situ experimental trial

Animals, diets and experimental design

The study was conducted at Bangladesh Livestock Research Institute (BLRI) research farm, Savar, Dhaka. Four local Pabna rumen fistulated bulls of an average live weight of 310±13 Kg fitted with rumen cannulae (14 cm diameter and 9 cm length) were used for this experiment in a 4X4 Latin Square Design for a period of 21 days for each. The nylon bags technique was applied to determine the dry matter (DM) and crude protein (CP) degradability of three herbs of moringa (MH), pineapple waste (PwH), plantain (PH), and the mix of the three herbs (MxH) at a ratio of 1:1:1, according to Orskov and McDonald, 1979, using nylon bag (7x16 cm) with pore size 45 μm. Approximately 3g of dried herb sample each was weighed previously tied to the nylon bags and incubated into the rumen of the fistulated bulls for a period of 4, 8, 16, 24, 48, and 72 hrs. Immediately after the removal of bags from the rumen, bags were washed in cold tap water until rinse were clear and dried in a forced air oven at 60^{0}C for 48 h (Karsli and Russell, 2002). The bags were weighed and the remaining residues were analyzed for DM and N concentrations according to AOAC, 2005. An additional bag from each treatment was directly washed with cold tap water to estimate 0 h incubation and dried in an oven at 60^0C for 48 h for determination of DM and N concentrations. The procedure was followed according to described by Ørskov and McDonald, 1979. The rumen fistulated animals of this experiment were allocated to a maintenance ration composed of Napier and German grass offered *ad libitum* basis with mixed concentrate mixture of wheat bran, mustard oil cake, Kheshari bran, di-calcium phosphate, and common salt at the rate of 1.0% of the live weight. Individual pens were used to keep the animals, and *ad libitum* water was supplied. Before the start of the study, the bulls were allowed a 10 days adaptation period for diet adjustment.

Calculation of DM and CP degradability

Dry matter disappearance (DMD) and crude protein disappearance (CPD) were estimated as follows (Osuji et al., 1993):

$$DMD= \frac{\text{(DM in feed sample -DM in residue)}}{\text{(DM in feed sample)}} \times 100$$

$$CPD= \frac{\text{(CP in feed sample –CP in residue)}}{\text{(CP in feed sample)}} \times 100$$

Degradability (Y) of DM and CP were calculated using the equation of Orskov and McDonald, 1979:

$Y = a + b\ (1 – e^{-ct})$

Where Y is the Cumulative degradation of dry matter at a given time, a is the soluble fraction, b is the insoluble but potentially degradable fraction, c is the degradation rate constant of the b fraction, t is the degradation time (0, 4, 8, 16, 24, 48 and 72 h) and e is the base for the logarithm.

Potential degradability (PD) was determined by the equation:

PD = a + b

Effective degradability (ED) for DM and CP was estimated following the method of Ørskov and McDonald, 1979:

$$ED = a + \frac{b \times c}{c + k}$$

Where a is the soluble fraction, b is the insoluble but potentially degradable fraction, c is the degradation rate constant of the b fraction, and k is the rumen outflow/passage rate (a passage rate assumed to be 0.02 and 0.05 per hour).

***In Vitro* Trial**

The experiment was conducted at Bangladesh Livestock Research Institute (BLRI), Savar.

Preparation of buffer solution

The buffer medium was created using the following ingredients in accordance with Asanuma et al., 1999; (i) Dipotassium phosphate - 0.45g/L; (ii) Monopotassium phosphate - 0.45g/L; (iii) Ammonium sulfate - 0.9 g/L; (iv) Sodium chloride - 0.9 g/L; (v) Calcium chloride dehydrate - 0.12g/L; (vi) Magnesium sulfate heptahydrate - 0.19g/L; (vii) Trypticase Peptone - 1.0g/L; (viii) Yeast extract - 1.0g/L and (ix) Cysteine hydrochloride - 0.6g/L.

Rumen liquor and total gas collection

The rumen fluid was collected from the four fistulated bulls reared at the BLRI research farm. After collection, rumen fluid was placed directly into pre-warmed thermo flasks before morning feeding and taken immediately to the laboratory. It was then filtered through a four-layer of cheesecloths and placed in a glass bottle with a cap and maintained at 39°C. Before combining with the buffer, the rumen fluid bottle was viciously shaken by hand. The rumen fluid was combined in a 1:3 rumen fluid: buffer ratio with a previously prepared buffer medium (pH 6.9) to create the inoculant. A steady flow of N_2 gas was used to anaerobically transfer 100 mL of buffered rumen fluid to 160 mL serum vials that contained 0.2 g of the herb substrate (0.1 mm in size). The serum bottles were then sealed with rubber septum stoppers and aluminum caps before being incubated at 39°C for 2, 4, 8, 16, 24, and 48 h in a 120-rpm shaking incubator (Hattori and Matsui, 2008). For each incubation time, three replicates per experimental treatment were used. For each incubation run, three bottles without substrate were also included as blanks. A 100 ml glass syringe was used to measure and record the volume of gas produced during the incubation process.

The organic matter digestibility (OMD) and metabolizable energy (ME) were estimated according to the method described by Menke et al., 1979 and Menke and Steingass, 1988:

$OMD\ (\%) = 14.88 + (0.889 \times Gv) + (0.45 \times CP) + (0.0651\times XA)$

$ME\ (MJ/kg\ DM) = 2.20 + (0.136\times G_v) + (0.0057\times CP) + (0.00029 \times EE)$

Where, GV, CP, EF, and XA were the total gas volume (ml/200 mg DM), crude protein, ether extract, and ash (g/kgDM) respectively of the incubated samples.

The short-chain fatty acid (SCFA) was estimated with the following equation adopted by Blümmel et al., 1999;

SCFA=0.0239×Gv-0.0601

Where, Gv was 48-hour net gas production (ml/200 mg DM)

Chemical analysis of shade dried herbs

To determine the proximate components of the three herbs and their mix, samples were dried in an air-forced oven at 65^{0}C for 48 hours and ground with a 1mm sieve for proximate analysis. According to the AOAC, 2000 analysis procedures, the dry matter (DM) (105^{0}C for 24 hours), crude protein (CP), ether extract (EE), and ash were all analyzed. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined using the Van Soest et al., 1991 method.

Statistical Analysis

In situ DMD and CPD were analyzed by using the Neway Excel program. Statistical data were analyzed using the SPSS 20.0 statistical program.

In the *in vitro* trial, data on IVOMD was used in a line chart and fitted in regression in an Excel program. The statistical model used for the analysis of variance was as follows:

$Y_{ijk}= \mu + \alpha_i+ \beta_j + e_{ijk}$

Where Y_{ijk} is the observation, μ is the overall mean, α_i is the treatment effect, β_j is the block effect and e_{ijk} is the error effect.

Duncan's Multiple Range Test (DMRT) was also done to compare the treatment means for different parameters (Steel and Torrie 1980).

Results

Chemical composition of shade-dried herbs

The chemical composition of three herbs (Moringa, Pineapple waste, and Plantain) and their mixed samples were shown in Table 1. The dry matter and CP content of all three shade-drying herbs ranged from 88.44 to 89.67% and 7.06 to 17.75 %, respectively. ADF and NDF content of the herbs ranged from 26.03 to 32.88% and 37.33 to 58.41%, respectively. EE and ash content ranged from 2.69 to 3.39% and 8.17 to 14.16%, respectively.

Table 1. Chemical composition (Mean ± SD) of shade-dried herbs

Items	DM %	Composition of herbs on a DM basis (%)					
		OM	CP	ADF	NDF	EE	Ash
MH	89.06±0.25	91.59±0.96	17.75±1.27	32.88±0.69	48.4±1.25	3.39±0.34	8.41±0.96
PwH	88.44±0.72	91.83±0.33	7.06±0.26	30.79±0.22	58.41±1.09	2.69±0.03	8.17±0.33
PH	89.67±0.56	85.84±0.27	14.5±0.79	26.03±0.32	37.33±0.45	2.72±0.01	14.16±0.27
MxH	89.05±0.11	89.76±0.18	13.1±0.03	29.9±0.23	48.04±0.09	2.93±0.16	10.24±0.18

MH, Moringa herb; PwH, Pineapple waste herb; PH, Plantain herb; MxH, Mixed of three herbs; DM, Dry matter; OM, Organic matter; CP, Crude protein; ADF, Acid detergent fiber; NDF, Neutral detergent fiber; EE, Ether extract; Values are (Mean ± SD) of the triplicate sample.

Table 2. Ruminal dry matter disappearance of herbs

Disappearance (%)	Incubation Time (h)						
	0	4	8	16	24	48	72
MH	22.14[a]	30.69[a]	44.07[b]	52.74[b]	56.20[b]	61.03[b]	71.28[c]
PwH	22.84[b]	31.76[b]	37.36[a]	46.21[a]	51.08[a]	56.45[a]	63.97[a]
PH	26.49[d]	36.90[d]	46.06[b]	59.03[d]	66.39[d]	70.46[d]	75.50[d]
MxH	24.98[c]	33.91[c]	44.91[b]	55.89[c]	62.90[c]	66.88[c]	68.75[b]
SEM	0.46	0.66	0.91	1.20	1.50	1.40	1.00
P Value	0.001	0.001	0.001	0.001	0.001	0.001	0.001

[a, b, c & d] Mean in the same column with different superscripts differ significantly (P<0.05); SEM, Standard error of the mean; MH, Moringa herb; PwH, Pineapple waste herb; PH, Plantain herb; MxH, Mixed of the three herbs.

In situ ruminal dry matter disappearances

The ruminal DM disappearance of different herbs was shown in Table 2. The result showed that ruminal disappearances differed significantly (p<0.001) among herbs at all incubation periods. PH had the greatest disappearance in the rumen from 4-72h whereas PwH had the lowest DM disappearance from 8-72h.

In situ dry matter degradability characteristics

The dry matter degradability of different herbs was shown in Table 3. Results showed that degradation parameters (a, b, c, PD, and ED) for DM varied significantly (P<0.05) among different herbs. The highest DM rapidly degradable fraction (a) was found in MxH and the lowest in MH whereas the slowly degradable fraction (b) was highest in MH and lowest in PwH among herbs. The potential (PD) and effective (ED) DM degradability were highest in PH and the lowest in PwH at 0.02 and 0.05 rates of passage per hour.

In situ ruminal crude protein disappearances

The ruminal CP disappearance of different herbs were shown in Table 4.

Table 3. *In situ* dry matter degradability characteristics of herbs

Herbs	DM Degradability (%)					
	a	b	c	PD	ED (K=0.02)	ED(k=0.05)
MH	22.14[a]	49.14[c]	0.07[b]	71.28[c]	60.39[b]	50.84[b]
PwH	22.84[b]	41.13[a]	0.02[a]	63.97[a]	47.18[a]	37.93[a]
PH	26.49[d]	49.01[c]	0.11[d]	75.50[d]	68.23[d]	60.63[d]
MxH	24.98[c]	43.77[b]	0.10[c]	68.75[b]	61.74[c]	54.62[c]
SEM	0.46	0.91	0.008	1.08	1.97	2.15
P Value	0.001	0.001	0.001	0.001	0.001	0.001

[a, b, c & d] Mean in the same column with different superscripts differ significantly (P<0.05); SEM, Standard error of the mean; MH, Moringa herb; PwH, Pineapple waste herb; PH, Plantain herb; MxH, Mixed of the three herbs; a, soluble fraction; b, slowly degradable fraction; c, rate of degradation; ED, Effective degradability; PD, Potential degradability.

Table 4. *In situ* ruminal crude protein disappearance of herbs

Disappearance (%)	Incubation Time (h)						
	0	4	8	16	24	48	72
MH	14.91[b]	25.15[b]	39.34[d]	49.03[d]	55.80[c]	61.90[c]	68.84[c]
PwH	13.04[a]	25.99[bc]	33.73[b]	38.93[a]	46.76[a]	55.32[a]	57.27[a]
PH	16.13[c]	23.34[a]	27.16[a]	40.91[b]	52.12[b]	59.25[b]	64.08[b]
MxH	17.92[d]	26.87[c]	36.35[c]	46.13[c]	51.68[b]	58.75[b]	67.71[c]
SEM	0.47	0.37	1.16	1.04	0.84	0.61	1.17
P Value	0.001	0.001	0.001	0.001	0.001	0.001	0.001

[a, b, c & d] Mean in the same column with different superscripts differ significantly (p < 0.05); SEM, Standard error of the mean; MH,Moringa herb; PwH, Pineapple waste herb; PH,Plantain herb; MxH, Mixed of the three herbs.

Table 5. *In situ* crude protein degradability characteristics of herbs

Herbs	CP Degradability (%)					
	a	b	c	PD	ED (K=0.02)	ED(k=0.05)
MH	14.91[b]	53.93[d]	0.051[b]	68.84[d]	53.64[b]	42.14[b]
PwH	13.04[a]	44.23[a]	0.03[a]	57.27[a]	41.88[a]	31.99[a]
PH	16.13[c]	47.95[b]	0.09[d]	64.08[b]	55.48[c]	47.13[c]
MxH	17.92[d]	49.78[c]	0.08[c]	67.71[c]	58.31[d]	49.4[d]
SEM	0.470	0.910	0.005	1.170	1.620	1.730
P Value	0.001	0.001	0.001	0.001	0.001	0.001

[a, b, c & d] Mean in the same column with different superscripts differ significantly (P<0.05); SEM, Standard error of the mean; MH, Moringa herb; PwH, Pineapple waste herb; PH, Plantain herb; MxH, Mixed of the three herbs; a, soluble fraction; b, slowly degradable fraction; c, rate of degradation, ED, Effective degradability; PD, Potential degradability.

Table 6. Cumulative gas production of different herbs at different incubation hours

Herbs	Cumulative gas production (ml/200mgDM) at different hours					
	2h	4h	8h	16h	24h	48h
MH	6.71[a]	17.02[b]	26.66[b]	38.14[c]	44.95[b]	56.18[c]
PwH	9.17[c]	20.73[c]	24.54[a]	31.94[a]	40.54[a]	46.93[a]
PH	7.66[ab]	14.61[a]	27.73[b]	36.48[bc]	47.86[c]	56.63[c]
MxH	7.96[b]	17.85[b]	26.03[ab]	35.01[b]	43.81[b]	52.96[b]
SEM	0.29	0.68	0.39	0.73	0.81	1.18
P Value	<0.001	<0.001	0.003	<0.001	<0.001	<0.001

[a, b & c] Mean in the same column with different superscripts differ significantly (P<0.05); SEM, Standard error of the mean; MH, Moringa herb; PwH, Pineapple waste herb; PH, Plantain herb; MxH, Mixed of the three herbs.

Table 7. IVOMD, ME, and SCFA of different herbs

Herbs	OMD %	ME (MJ/kg DM)	SCFA (mmol)
MH	66.17[c]	9.94[c]	1.28[c]
PwH	57.45[a]	8.62[a]	1.06[a]
PH	66.8[c]	9.99[c]	1.29[c]
MxH	63.22[b]	9.48[b]	1.21[b]
SEM	1.12	0.16	0.02
P Value	<0.001	<0.001	<0.001

[a, b & c] Mean in the same column with different superscripts differ significantly (P<0.05); SEM,Standard error of the mean; MH, Moringa herb; PwH, Pineapple waste herb; PH, Plantain herb; MxH, Mixed of the three herbs; OMD, Organic matter digestibility; ME, Metabolizable energy ;SCFA, Short chain fatty acids.

Results showed that ruminal CP disappearances differed significantly (P<0.05) among herbs at all incubation periods. MH had the greatest CP disappearance in the rumen from 4-72h whereas PwH had the lowest CP disappearance at 72h.

In situ crude protein degradability characteristics

In situ CP degradability of different herbs was shown in Table 5. Results showed that degradation parameters (a, b, c, ED, and PD) for CP varied significantly (P<0.05) among different herbs. The highest CP rapidly degradable fraction was found in MxH and the lowest in PwH whereas the slowly degradable fraction (b) was highest in MH and lowest in PwH among herbs. The potential (PD) and effective (ED) CP degradability were highest in MH and MxH, respectively whereas the lowest in PwH at 0.02 and 0.05 rates of passage per hour.

In vitro gas production

The *in vitro* gas production of MH, PwH, PH, and MxH up to 48 hours of incubation was shown in Table 6. The result showed that the cumulative gas production was increased with increasing the periods of incubation for all herbs. The highest gas production at 2h was seen in PwH followed by MxH, PH, and lowest in MH. After 48 h, significantly (P<0.05) highest gas production was found in both PH and MH followed by PwH and MxH substrate.

In vitro OM digestibility (IVOMD), energy value, and short-chain fatty acid (SCFA)

In vitro organic matter digestibility (IVOMD), metabolizable energy (ME) concentration and short-chain fatty acid (SCFA) of MH, PwH, PH, and MxH were presented in Table 7. The result showed that IVMOD and ME were significantly (P<0.05) higher in both MH and PH and lower in PwH. Short-chain fatty acids were also higher in MH & PH and lower in PwH.

Discussion

Chemical composition of herbs

The nutrient composition of herbs varied due to differences in agro-climatic conditions, the physiological stage of trees, and the different stages of maturity of leaves (Foidl et al., 2001; Moyo et al., 2011). The herbs of moringa are excellent fodder, with high crude protein (CP) concentrations stated by Fadiyimu et al., 2010; Ndemanisho et al., 2007 and on a dry matter basis, the crude protein content of moringa leaf ranges from 23 to 30.3% (Wu et al., 2013). The CP content of the present findings was lower (17.75%) than the above-mentioned. The incorporation of soft twigs and leaves in moringa leaf meal instead of leaves alone decreased 22% CP concentration in the meal observed by Kakengi et al., 2005; Falowo et al., 2018; Méndez et al., 2018. Several authors reported that the CP content of pineapple peel waste ranged from 6.04 to 8.7% (Suksathit et al., 2011; Paengkoum et al., 2017; Wittayakun et al., 2015; Paengkoum et al., 2013; Dahlan, 2013) which was similar to the present findings (7.06%). Guil-Guerrero, 2001 and Kara et al., 2015b found that the crude protein content of PH ranged from 11 to 16% in different phenological stages which was similar to the present findings (14.5%). On the other hand, Harrington et al., 2006 found that the CP content of PH was 28.3% which was higher than the present findings. A wide variation in ADF, NDF, and EE content of moringa (Kakengi et al., 2005; Mendieta-Aracia et al., 2013; Makkar and Becker, 1997; Jongrungruangchock et al., 2010; Manh et al., 2005), pineapple waste (Negesse et al., 2009 and Dahlan, 2013; Paengkoum et al., 2013; Ikhimioya et al., 2005; Wittayakun et al., 2015; Asaolu et al., 2016; Braga et al., 2016; Buliah et al., 2019) and plantain herbs (Beck et al., 2020; Kara et al., 2018; Redoy et al., 2020). They reported that ADF of moringa herbs ranged from 7.9 to 68.4%, NDF ranged from 11.1 to 68.40% and EE ranged from 2.26 to 10.91% of dry matter. The ADF content of pineapple waste herbs ranged from 20.8 to 50.3%, NDF ranged from 45 to 67.7% and EE ranged from 1.18 to 2.9% of DM. They also reported that plantain herbs ADF ranged from 25.84 to 32.8%, NDF ranged from 30.33 to 46.33% and EE ranged from 1.79 to 2.65% of DM. The variations of oil components could be related to plant parts, season and environmental conditions, method of harvesting, geographical zone, pedological conditions, and the method used to isolate the plant product (Gil et al., 2002).

In situ ruminal dry matter disappearances and degradability characteristics of herbs

Present findings showed that PwH had shown a slower rate of DM disappearance than MxH, MH, and PH which could be due to the structure of the cell wall and the chemical composition of feedstuffs mainly higher fiber content. This was consistent with Waghorn et al., 2003 who stated that lignification restricts microbial access to structural polysaccharides in the cell wall resulting in slower digestion. Pineapple waste and mixed herbs contained high levels of NDF and ADF, resulting in a low rate of disappearance of the soluble fraction of DM. The DM disappearance of the plantain and moringa herbs was observed higher in the present study. This may be due to an increase in the energy supply to rumen microorganisms and the high disappearance of DM percentage in the rumen is nutritionally advantageous (Chaves et al. 2006a). Dry matter degradability of herbs increased with increasing time of incubation. The present findings illustrate that the degradation parameters of a, b, c, ED, and PD of DM were higher ($P<0.05$) in plantain and moringa herbs than others, which could be mainly associated with structural, chemical composition and anti-nutritional factors in the feedstuffs. Bashar et al., 2016 studied DM degradable kinetics of moringa foliage for different cutting diameters and found that a, b, and c ranged from 20.9-23%, 45.26-47.43%, and 0.08-0.13%, respectively which was in accordance with the present findings but effective degradation (51.79-54.22%) was lower than the present findings. Ikhimioya et al., 2005 observed that pineapple waste DM degradable kinetics of a, b, c, and ED were 33.88, 34.40, 0.01, and 43.29%, respectively which was higher in degradation fraction (a), but slightly lower in degradable fraction (b) and effective degradation (ED) of the present findings. Box et al., 2018 observed that plantain herbs DM degradable kinetics of a, b, c, and ED was 23.7, 68.7, 0.11, and 68.7%, respectively which was slightly lower in degradation fraction (a), but higher in degradable fraction (b) whereas similar as effective degradation (ED) of the present findings.

In situ ruminal crude protein disappearances and degradability characteristics of herbs

The process of digestion was seen an uptrend over incubation time and the degradability was significantly different among herbs. Present findings showed that PwH had shown a slower rate of CP disappearance than PH, MxH, and MH which could be due to the structure of the cell wall and the chemical composition of feedstuffs. These findings agreed with Waghorn et al., 2003 who stated that lignification limits microbial access to structural polysaccharides in the cell wall resulting in slower digestion. The lower crude protein degradability at the beginning of incubation is important from a nutritional view as lower degradability at the beginning of incubation indicates greater bypass protein that will be utilized in the duodenum (Barry and Manley, 1986; Kumar and D'Mello, 1995). The present findings illustrate that pineapple waste and moringa herbs showed lower CP degradability at the beginning of incubation than other herbs. The degradation profile can be affected by the amount of fiber in feeds. Higher fibers in feed protect nitrogen bound to the fiber which decreases the amount of nitrogen available to rumen microorganisms (Kendall et al., 1991). Seradj et al., 2019 observed that moringa leaves harvested at 40 (M40), days after pruning, the CP degradable kinetics of a, b, c, and ED were 34.7, 60.9, 0.14, and 78.3%, respectively which was higher than the present findings. Ikhimioya et al., 2005 observed CP degradable kinetics of a, b, c, and ED were 14.06, 12.56, 0.031, and 18.79%, respectively which was lower than the present findings. Whereas, Braga et al., 2016 observed that pineapple by-products CP degradable kinetics of a, b, c, and ED were 17.87, 73.06, 3.7, and 48.53%, respectively which was higher than the present findings. Box et al., 2018 observed that plantain herb CP (Nitrogen) degradable kinetics of a, b, c, and ED were 10.3, 85.5, 0.85, and 60%, respectively which was slightly lower in degradation fraction (a) and effective degradation (ED) but higher in degradable fraction (b) of the present findings. There are numerous factors that affect the amount of CP in feeds that will be degraded in the rumen, the two most important considerations being the proportional concentrations of NPN and true protein, and the physical and chemical characteristics of the proteins that comprise the true protein fraction of the feedstuffs (NRC, 2001).

In vitro gas Production, OM digestibility (IVOMD), energy value, and short chain fatty acid (SCFA) of different herbs

The highest volume of gas production in the present study was observed in moringa and plantain herb which could be due to high crude protein or bioactive compounds that enhanced microbial multiplication in the rumen, which in turn determines the extent of fermentation. Higher potential gas production can greatly contribute to the supply of energy through the creation of short-chain fatty acids (Remesy et al., 1995). Karim, 2012 studied *in vitro* gas production of Moringa oleifera leaves and obtained 32.58, 60.72, 97.30, and 120.52 ml/g gas at 3h, 6h, 9h, and 12h of incubation, respectively which was lower than the present findings. Pineapple waste herbs showed a lower extent of gas production than other herbs at all stages of incubation. The chemical components of NDF and ADF may slow down the speed of substrate fermentation (Fievez et al., 2005). Fasae and Alokan, 2006 observed that a decrease in the rate and extent of gas production of some shrubs is due to their high contents of lignin and tannin through increasing adverse environmental conditions as incubation time progresses. Ranges of gas production characteristics as obtained in this study may be influenced by variations in CP, NDF, and ADF contents in feed ingredients as reported by Njidda and Nasiru, 2010. According to Nsahlai et al., 1994 and Larbi et al., 1998, CP and gas production rates exhibited a positive relation, while NDF and ADF and gas production rates and extent exhibited negative relationships. De Boever et al., 2005 stated a positive correlation between starch and gas production and a negative correlation between NDF content and gas production. Differences in botanical fraction (i.e., tops, leaf, stem), as well as concentrations of anti-nutritional components such as tannin, could also be responsible for variation in cumulative gas production (Larbi et al., 2011; Njidda and Nasiru, 2010). The differences in organic matter digestibility among feeds could be due to differences in plant species, morphological fraction, environment, or stage of maturity which was agreed with the observation of Fedorak and Hrudey, 1984. The highest IVOMD was obtained in PH (66.80%) and MH (66.17) and the lowest in PwH (57.45%). Aderinola and Binuomote, 2014 found that the IVOMD of moringa foliage was 50.22% which was lower than the present findings. A wide range of predicted ME values were noted among different species and agronomic conditions at different locations. The findings of Sultana et al., 2015; Njidda and Nasiru, 2010 and Aderinola and Binuomote, 2014, of the ME value of moringa were 9.60, 4.33, and 6.59 MJ Kg-1 DM respectively which was lower than the present findings. The present findings of short-chain fatty acid (SCFA) predicted by *in vitro* gas production among herbs differed significantly (P<0.01). The variations of SCFA among feeds could be attributed to differences in plant species, morphological fraction, environment, or age of plants as also had been observed by Fedorak and Hrudey, 1984. The close association between SCFA and gas production *in vitro* technique was reported by Getachew et al., 2002.

Conclusions

Plantain and moringa herbs showed better potential and effective degradability and also higher organic matter digestibility and metabolizable energy comparable to pineapple waste herbs. It may be concluded that the evaluation of the degradability of moringa, pineapple waste, and plantain herbs using *in situ* and *in vitro* gas production techniques provides valuable information for optimizing their utilization in ruminant nutrition.

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Author's contribution

MA Alam executed the research trial and lab analysis, data analysis, manuscript writing, and editing; ZH Khandaker was involved in supervision and planning of the research; MAI Talukder was involved in supervision and funding acquisition; M Hasan-Al-Sharif involved lab analysis and manuscript writing; M Al-Mamun was conceptualizing the research, supervision, involved in the editing and critical checking of the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Data Availability

All the necessary data used in this research are available as per the authorization of the authors.

Ethical approval

All experimental protocols related to animals were approved by the Institutional Committee for Animal Use and Ethics of Bangladesh Livestock Research Institute (BLRI).

Consent to Participate

The authors provide full consent to participate as per need.

Consent for Publication

All the author has fully agreed to publish this research in Bangladesh Journal of Animal Science.

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