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Original Article

Nutritional Evaluation of Conventional Feedstuffs for Ruminants using InVitro Gas Production Technique

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ABSTRACT

The use of crop residues and agricultural by-products in animal feeding is a very common practice in Myanmar. Nutritive values and fermentation characteristics of natural grass (NG), lablab bean stalk (LLB), butter bean residue (BBR), sesame residue (SR), dried sorghum stover (DSS) and fresh sorghum stover (FSS) were evaluated through *in vitro* gas production method. The crude protein (CP) content of Lablab bean stalk (LLB) was significantly higher than those of other feedstuffs with the least CP obtained from dried sorghum stover (DSS). The contents of NDF, ADF and EE ranged from 58.95% to 80.03%, 36.63% to 67.76% and 2.15% to 2.89%, respectively. The cumulative gas volumes at 24 hrs ranked from the highest to the lowest; BBRD, NG, SRD, LLBF, SSD and SSF. However, the gas volume of SSD was significantly higher ($P<0.05$) than those of other feedstuffs at the 96 hr. of incubation time. The highest gas production rate “c” was found in SRD and the lowest was in SSD. While the fermentation of insoluble fraction “b” of SSD was significantly higher ($P<0.05$) than those of other feedstuffs, the LLBF was the highest in the fermentation of quickly soluble fraction “a”. The estimated total carbohydrate (TC), non fibre carbohydrate (NFC), metabolizable energy (ME), organic matter digestibility (OMD), short chain fatty acid (SCFA) and methane gas concentration are significantly different ($P<0.05$) among the experimental feedstuffs. According to the findings of this study, all of these conventional feedstuffs have the good potential to be used as ruminant feed.

Keywords: Conventional feedstuff, Nutritive values, *in vitro* gas production.

INTRODUCTION

The use of crop residues and agricultural by-products in animal feeding is a very common practice in Myanmar. Evaluating the nutritive value of these available feed resources are important as these could make an important contribution to the nutrition of livestock. Tesfay

(2014) pointed out that the dairy farmers at the peri-urban area rely on the dry and green roughages for the feed of their animals. Fermentation characteristics of feedstuffs in buffered rumen fluid can be studied using *in vitro* techniques using syringes (Menke *et al*., 1979; Blümmel *et al.*, 1997). These techniques stimulate the rumen fermentation process and they have been used to evaluate the potential of feed to supply nutrients to ruminants.

The *in vitro* gas production technique does not require sophisticated equipment and the large numbers of samples can be incubated and analyzed at the same time. This method has been successfully applied in the aspects of feed evaluation, including organic matter digestibility (OMD), metabolizable energy (ME) (Menke *et al*., 1979: Menke and Steingass, 1988), short chain fatty acid (SCFA) (Makkar, 2005), kinetics of fermentation (Ørskov and McDonald, 1979) and the efficiency of microbial protein synthesis (Makker and Becker, 1996).

Moreover, the *in vitro* gas production method can be used to examine animal waste components that impact the environment and develop appropriate mitigations. Therefore, this experiment was intended to evaluate the nutritive values of conventional feedstuff using *in vitro* gas technique.

MATERIALS AND METHODS

Experimental feedstuffs

All experimental feedstuffs, natural grass (NG), lablab bean stalk (LLB), butter bean residue (BBR), sesame residue (SR), dried sorghum Stover (DSS) and fresh sorghum Stover (FSS) were collected from the central dry zone (CDZ) of Myanmar.

Chemical Analysis

All ground samples of conventional feedstuffs were collected for the chemical analysis. All ground samples were analyzed for dry matter (DM), organic matter (OM) by the method described by AOAC (1990) and neutral detergent fibre (NDF) and acid detergent fibre (ADF) by Goering and Van Soest (1970). Nitrogen contents were analyzed by using Kjeldahl method (Foss 2020 digester and Foss 2100 Kjeltec distillation unit) and crude protein (CP) is calculated as 6.25 × N (AOAC, 1990).

In Vitro Gas Production

Prior to the collection of rumen fluid, the animal was fed with 14 kg of 4% urea treated rice straw and 1.2 kg of sesame meal twice a day for 14 days for adaptation. Rumen fluid was collected from fistulated bull (300 Kg Body Weight) before morning feed. The procedure for *in vitro* gas production was as reported by Menke and Steingass (1988). Rumen fluid was mixed with buffer medium at ratio of 1:2 (v/v) under a continuous stream of carbon dioxide (CO_2). Substrate weighing 200 mg was introduced into the 120 ml calibrated syringe and followed by adding of 30 ml inoculums. Blanks sample were also determined for gas production. Incubation was carried out at 39˚C and gas production was read at 1, 3, 6, 12, 24, 48, 72 and 96 hrs.

In order to estimate methane production by the substrate and immediately after evacuation from the incubator, 4 ml of NaOH (10 M) was introduced using 5 ml capacity syringe as reported by Fievez *et al.* (2005). The content was inserted into the silicon tube, which are fastened to the 120 ml capacity syringe. The clip was then opened while the NaOH was gradually released. The content was agitated while the plunger began to shift position to occupy the vacuum created by the absorption of CO_2. The volume of methane was read on the calibration. The extent and rate of fermentation on gas production is determined by exponential model of Ørskov and McDonald (1979);

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

Where:

a = The gas production (ml) from quickly soluble fraction

b = The gas production (ml) from insoluble fraction

c = The gas production rate constant for the insoluble fraction

a+b = Potential gas production (ml)

t = Incubation time (h)

Y = Gas production at time t.

The total carbohydrate (CT), non fibre carbohydrate (NFC), short chain fatty acid (SCFA), organic matter digestibility (OMD) and metabolizable energy (ME) values in experimental feedstuff were calculated using equation as below:

TC = 100 – (CP + EE + Ash) (NRC, 2001)

NFC = 100 – (NDF + CP + EE + Ash) (NRC, 2001)

SCFA (mmol) = 0.0222Gp – 0.00425 (Makkar, 2005)
OMD (%) = 14.88 + 0.889Gp+ 0.458CP+0.0651XA (Menke and Steingass, 1988)
ME (MJ/Kg DM) = 2.20 + 0.136Gp + 0.057CP (Menke and Steingass, 1988)

Where;
CP = % crude protein in DM
Gp = gas production from 200mg sample at 24 hrss
XA = % ash in DM

Statistical Analysis

The data were subjected to the analysis of variance (ANOVA) and the significance of differences between treatments means were compared by Duncan's Multiple Range Test (DMRT) (Steel and Torrie, 1980) using SPSS (version 16.0) software.

RESULTS AND DISCUSSIONS

The chemical composition (%) of conventional feedstuffs is presented in Table 1. The result shows that there are wide variations in crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) content. The CP content of Lablab bean stalk (LLB) was significantly higher than those of other feedstuffs with the least CP obtained from dried sorghum stover (DSS). The contents of NDF, ADF and EE ranged from 58.95 to 80.03, 36.63 to 67.76 and 2.15 to 2.89, respectively. The variation observed in the chemical composition (%) of experimental feedstuffs could be due to many factors such as stage of growth, maturity, species or variety (Von Keyserlingk *et al*., 1996; Agbagla-Dohanni *et al*., 2001; Promkot and Wanapat, 2004), drying method, growth environment (Mupangwa *et al*., 1997) and soil types (Thu and Preston, 1997).

Table 1: Chemical composition (%) of conventional feedstuffs

Description	DM	OM	CP	NDF	ADF	EE
NG	41.32[d]	89.39[d]	8.18[c]	68.62[c]	36.63[e]	2.39[b]
LLBF	20.62[e]	88.76[d]	19.48[a]	58.95[e]	41.73[d]	2.89[a]
BBRD	83.60[a]	96.73[a]	5.86[d]	77.50[a]	67.76[a]	2.48[b]
SSRD	74.32[c]	92.24[c]	8.50[c]	61.97[d]	56.77[b]	2.15[c]
SSD	77.76[b]	93.95[b]	3.29[e]	80.03[a]	54.52[b]	1.67[d]
SSF	21.63[e]	89.21[d]	9.59[b]	74.81[b]	48.82[c]	2.17[c]
SEM	6.39	0.72	1.23	1.91	2.54	0.09
P Value	0.001	0.001	0.001	0.001	0.001	0.001

[a, b, c, d, e:]Means within the same column with various superscripts are significantly different. SEM: standard error mean

Wide variations were also observed in the cumulative gas production at different incubation times (Table 2). The result indicates that the cumulative gas volumes at all incubation times were significantly different ($P<0.05$). The gas volumes at 24 hrs ranked from the highest to the lowest; BBRD, NG, SRD, LLBF, SSD and SSF respectively. However, the gas volume of SSD was significantly higher ($P<0.05$) than those of other feedstuffs at the 96 hrs of incubation time

Table 2: Cumulative gas production of conventional feedstuff at the different incubation times

Description	Incubation times (hrs)							
	1	3	6	12	24	48	72	96
NG	9.67[ab]	12.82[a]	17.07[a]	24.09[ab]	33.73[ab]	42.96[a]	46.28[a]	47.47[ab]
LLBF	10.97[a]	13.68[a]	17.22[a]	22.76[ab]	29.56[bc]	34.85[b]	36.28[c]	36.66[e]
BBRD	9.24[ab]	13.19[a]	18.30[a]	26.16[a]	35.52[a]	42.37[a]	44.02[ab]	44.42[bcd]
SRD	7.99[b]	11.63[a]	16.29[a]	23.32[ab]	31.35[abc]	36.81[ab]	38.01[bc]	38.27[de]
SSD	3.72[c]	6.44[b]	10.25[b]	17.07[c]	27.96[c]	41.91[a]	49.51[a]	53.65[a]
SSF	2.70[c]	5.15[b]	9.30[b]	16.39[c]	26.83[c]	38.26[ab]	43.24[ab]	45.41[bc]
SEM	0.69	0.77	0.81	0.85	0.81	0.91	1.17	1.39
P Value	0.001	0.001	0.001	0.001	0.019	0.055	0.005	0.001

[a, b, c, d, e:]Means within the same column with various superscripts are significantly different. SEM: standard error mean

The gas production parameters (a, b, c) were shown in Table 3. Accordance with the table, the highest gas production rate "c" was found in SRD and the lowest was in SSD. While the fermentation of insoluble fraction "b" of SSD was significantly higher (P<0.05) than those of other feedstuffs, the LLBF was the highest in the fermentation of quickly soluble fraction.

The gas production from soluble fraction "a" of NG and LLBF were high when compared to other feedstuffs, probably a reflection of high level of soluble carbohydrate, conversely low level of insoluble carbohydrate (NDF and ADF). The higher gas production from insoluble fraction "b" was observed in SSD, possibly influenced by the low level of soluble carbohydrate fraction readily available to the microbial population and high level of insoluble carbohydrate. Deaville and Given (2001) reported that kinetics of gas production could be affected by carbohydrate fraction. Additionally, the highest protein content was found in LLBF and the lowest was in SSD. The protein fermentation does not lead to excessive gas production (Khazaal *et al.*, 1995).

Table 3: Kinetic fermentation of conventional feedstuff through the *in vitro* gas method

Description	*a*	*b*	*c*	*a+b*
NG	7.99[ab]	40.16[c]	0.0429[d]	48.15[b]
LLBF	9.49[a]	27.31[e]	0.0560[b]	36.80[d]
BBRD	7.09[b]	37.45[cd]	0.0593[ab]	44.54[bc]
SRD	5.98[b]	32.34[de]	0.0643[a]	38.31[cd]
SSD	2.32[c]	56.29[a]	0.0253[f]	58.61[a]
SSF	1.75[c]	46.56[b]	0.0346[e]	48.31[b]
SEM	0.64	2.09	0.003	1.69
P Value	0.0001	0.0001	0.0001	0.0001

[a, b, c, d, e, f:] Means within the same column with various superscripts are significantly different, *a*: gas production (ml) from quickly soluble fraction, *b*: gas production (ml) from insoluble fraction, *c*: gas production rate, *a + b:* potential gas production, SEM: Standard error mean

The fast gas production rate "c" observed in SRD and BBRD was probably influenced by the soluble carbohydrate fraction (NFC content) readily available to the microbial population. The low content of fibre (cellulose, hemicellulose and lignin) can facilitate the utilization of feed by ruminal microbes, which in turn might induce higher fermentation rates, therefore improving digestibility (Van Soest, 1994).

The gas production of feed in buffered rumen fluid is associated with feed fermentation and carbohydrate fraction (Sallam *et al.*, 2008). Low gas production of SSD in initial incubation times (24 hrs) compared to the other feedstuffs was resulted due to high content of slowly fermented carbohydrates (NDF) in SSD. This is in agreement with the finding of De Boever *et al.* (2005), who reported that gas production was negatively related with NDF content and positively with starch. However, at the late incubation time (96 hrs), the highest gas volume was found in SSD. It might be due to high level of NDF in SSD and it needs more time to attachment of microorganism.

The amount of total carbohydrate (TC), non fibre carbohydrate (NFC), metabolizable energy (ME), organic matter digestibility (OMD), short chain fatty acid (SCFA) and methane gas concentration of experimental feedstuffs are presented in Table 4. The estimated TC, NFC, ME, OMD, SCFA and methane gas concentration are significantly different (P<0.05) among the experimental feedstuffs.

The lowest reduction in methane gas concentration of LLBF could be due to low level of NDF and ADF which convert CO_2 and H_2 to acetate instead of CH_4 (Miller, 1995). This is consistent with the finding of Sallam *et al.* (2008). The estimated ME in this study were consistent with those obtained for the tropical feed sources and by-products (Akinfemi *et al.*, 2012) and lower than those obtained for the chickpea wastes (Maheri-Sis *et al.*, 2007). There was a positive correlation between ME calculated from *in vitro* gas production together with CP and fat content with metabolizable energy value of conventional feed measured in vivo (Menke and Steingass, 1988). The *in vitro* gas production method has been widely used to evaluate the energy value of several classes of feed (Getachew *et al*., 1999, 2002).

The least SCFA predicted for LLBF and SSD is due to a lower gas production which was evident in the first 24 hrs of incubation. Blummel *et al*. (1990) suggested that gas production from cereal straws and different class of feeds incubated *in vitro* in buffered rumen fluid was closely related to the production of SCFA which was based on carbohydrate fermentation. A high value of SCFA is an indication of energy availability to the animal. The digestibilities of organic matter (OMD) obtained in SRD, BBRD and SSF are high compare with other feedstuffs because the major carbohydrate of their feedstuffs is starch, which is fermented by amylolytic bacteria and protozoa (Kotarski *et al*., 1992), but did not support observation on the SSF sample in this study. This result implies that the microbes in the rumen and animal have high nutrient uptake.

Table 4: Estimated parameters of TC, NFC, ME, OMD, SCFA and Methane gas concentration (%) of conventional feedstuffs

Description	**TC (%)**	**NFC (%)**	**ME (MJ/ Kg DM)**	**OMD (%)**	**SCFA (mmol/ 200 mg DM)**	**Methane gas Concentration (%)**
NG	78.55[c]	10.03[bc]	6.45[cd]	44.00[c]	0.61[bc]	8.94[c]
LLBF	66.37[d]	7.71[c]	6.68[bc]	46.40[b]	0.55[d]	8.58[c]
BBRD	88.41[a]	11.22[b]	7.20[a]	48.22[ab]	0.76[a]	43.55[a]
SRD	81.58[b]	19.94[a]	7.24[a]	49.00[a]	0.74[a]	9.72[c]
SSD	88.99[a]	9.20[bc]	6.08[e]	40.89[d]	0.60[cd]	12.83[c]
SSF	77.45[c]	3.81[d]	6.91[ab]	47.89[ab]	0.67[b]	11.78[c]
SEM	1.85	1.24	0.09	0.64	0.02	2.71
P Value	0.001	0.001	0.001	0.001	0.001	0.001

[a, b, c, d, e:] Means within the same column with various superscripts are significantly different, TC: total carbohydrate, NFC: non-fibre carbohydrate, ME: metabolizable energy, OMD: organic matter digestibility, SCFA: short chain fatty acid, SEM: standard error mean

CONCLUSION

In this study, wide variations were occurred in the chemical composition (%), rate and extent of gas production and some estimated parameters of conventional feedstuffs. However, all of these conventional feedstuffs have the good potential to be used as ruminant feed.

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