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Comparison of *in vitro* Fermentation with *in situ* Degradation to Estimate Dry Matter Degradability and Energy Protein Synchronization of Roughage Based Diets

Research Article

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ABSTRACT

This study was aimed to evaluate the correlation between *in vitro* fermentation and *in situ* degradation parameters and to predict dry matter degradability and energy protein synchronization of roughage based diets. Different inclusion of roughage in diets [roughage 50% diet ($R_{50}D$), roughage 60% diet ($R_{60}D$), roughage 65% diet ($R_{65}D$) and roughage 70% diet ($R_{70}D$)] were used to determine *in vitro* and *in situ* parameters. The relationships between *in vitro* and *in situ* parameters were analyzed by simple linear regression. The gas volumes and fermentation kinetics of $R_{50}D$ and $R_{65}D$ were greater ($P<0.05$) than those of other diets, however the lesser values of partitioning factor for microbial protein synthesis efficiency was found in $R_{50}D$. Although the nutrient disappearances, degradation kinetics and effective degradability were greater ($P<0.05$) in $R_{50}D$ in compare with $R_{60}D$ and $R_{65}D$, the lowest ($P<0.05$) energy protein synchronization was observed in $R_{50}D$. The significant correlation ($P<0.05$) were observed in all regression equations of *in vitro* gas volumes and *in situ* nutrient disappearances. The *in situ* effective dry matter degradability and energy protein synchronization were correlated ($P<0.05$) with *in vitro* fermentation kinetics and some estimated parameters such as short chain fatty acid and partitioning factor for microbial protein synthesis efficiency. Among the correlations, the greater accuracy could be achieved by inclusion of two or more parameters in regression equation. The results showed that *in vitro* gas production technique has the potential to predict effective dry matter degradability and energy protein synchronization of roughage based diets.

KEY WORDS correlation, degradability, *in situ* degradation, *in vitro* fermentation, synchronization.

INTRODUCTION

The ruminal degradation of feed is very crucial to assess its nutritional status for ruminants. Degradability of various feeds can be determined by *in vivo* and *in situ* (nylon bag) methods. The rate and extent of nutrient degradation in the rumen can influence the synchronization of ruminal available energy and protein which is one of the conceptual methods to increase the efficiency of utilization of nutrients by ruminants and it can also be determined by *in situ* techniques. Feeding energy and protein synchronized feeds to ruminants has the potential to increase the efficiency of ruminal microbial protein synthesis, nitrogen usage, rumen fermentation, animal performance and to decrease urinary nitrogen excretion (Sinclair *et al.* 1993; Cole and Todd, 2008). The *in situ* technique has been used for many years to estimate the rate and the extent of nutrient degradation and synchronization (Mehrez and Ørskov, 1977), however it is laborious, time consuming and expensive (Cone *et al.* 2002). Otherwise, the *in vitro* gas production technique was

developed to estimate the rate and extent of nutrient fermentation and to evaluate the nutritive values of forages (Menke *et al*. 1979; Menke and Steingass, 1988), agro industrial by-products (Krishna and Gunther, 1987), various types of tropical feeds (Krishnamoorthy *et al*. 1995), compound feeds (Aiple *et al*. 1996), and tree and shrub legume forages (Makkar *et al*. 1999). Moreover, this method is less animal dependent, more appropriate for characterizing soluble or small particle feeds in comparison with *in situ* technique (Adesogan, 2002).

These two techniques, *in vitro* and *in situ*, are well correlated with animal performance (Ørskov, 1989), feed intake (Blümmel and Ørskov, 1993), microbial protein synthesis (Krishnamoorthy *et al*. 1991) and *in vivo* digestibility (Khazaal *et al*. 1993). Considering the advantages of gas production technique with its simplicity of use and the possibility of processing a large number of samples in a short time, it is important to find and valid correlations between *in situ* dry matter degradability and *in vitro* fermentation parameters (Valentin *et al*. 1999). Moreover, the relationships between *in vitro* fermentation parameters and *in situ* degradation parameters have been investigated by many researchers (Cone *et al*. 2002; Kamalak *et al*. 2005; Ozkan and Sahin, 2006); however most of researches are emphasized on feedstuffs and results somewhat inconsistent. Moreover the information concerning the relationships between *in vitro* fermentation and *in situ* degradation parameters of roughage based diets are also limited. Therefore, this study was aimed to investigate the correlation between *in vitro* fermentation and *in situ* degradation parameters and to predict dry matter degradability and energy-protein synchronization of roughage based diets.

MATERIALS AND METHODS

Feed and chemical analysis

The feedstuffs used in this study were rice straw, sorghum stover, grass, cotton seed cake, chickpea meal and broken rice which are commonly used as feed for dairy cows in Myanmar. The several inclusion rates of those feedstuffs were used in diet formulation to obtain different levels of roughage and concentrate in diets. The experimental roughage based diets are roughage 50% diet ($R_{50}D$), roughage 60% diet ($R_{60}D$), roughage 65% diet ($R_{65}D$) and roughage 70% diet ($R_{70}D$). As the chemical analysis, dry matter (DM), organic matter (OM), ether extract (EE) were analyzed by the method described by AOAC (1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed according to the method of Goering and van Soest (1970). All feeds were analyzed for nitrogen (N) by using Kjeldahl method (Fross 2020 digester and Foss 2100 Kjeltec distillation unit) and crude protein (CP) was calculated as 6.25 × N. The chemical compositions of experimental diets were ranged from 41.69% to 58.94% DM, 88.12% to 90.98% OM, 11.46% to 17.96% CP, 59.07% to 64.24% NDF, 34.74% to 47.98% ADF and 1.78% to 2.06% EE.

In vitro fermentation and measurements

Rumen fluid was obtained from a fistulated bull (320 kg BW) fed twice daily with a diet containing rice straw (60%) and concentrate (40%). The *in vitro* gas production method was done according to the procedures of Menke and Steingass (1988). Incubation was carried out at 39 ˚C and gas production was read at 1, 12, 24, 48 and 72 h. The rate and extent of gas production was determined by exponential model of Ørskov and McDonald (1979):

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

Where:
a= gas production (mL) from rapidly fermentable fraction.
b= gas production (mL) from slowly fermentable fraction.
c= constant rate of fermentation for the slowly fermentable fraction.
a + b= potential gas production (mL).
t= incubation time (h).
Y= gas production at time t.

After 24 h incubation, metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acid (SCFA) of experimental diets were calculated as below:

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

Where:
CP: % crude protein in DM.
Gp: gas production from 200mg sample at 24 h.
XA: % ash in DM.

For the analysis of partitioning factor (PF) for microbial protein synthesis, another incubation was conducted with sample weights increased from 200 mg to 500 mg so as to increase the mass of residue to minimize the analytical error. The double strength medium (40 mL) was incubated with 500 mg air-dry substrate. After 24 h of incubation, the contents were centrifuged (20000 g, 30 min, 4 ˚C) and the supernatant discarded. The pellet was washed with distilled water followed by centrifugation at least 3 times. The undigested substrate and microbial mass were dried in the oven

at 135 ˚C for 2 h. The residue was termed as apparent undigested residue. The residue was refluxed with neutral detergent solution (70 mL) for 1 h to determine NDF and NDF ash. From them the partitioning factor for microbial protein synthesis efficiency was calculated according to Makkar and Becker (1996) and it was described as mg of true digestible organic matter per ml of gas (true digestible OM (mg)/mL of gas).

In situ degradation and measurements

The *in situ* nutrient degradation was carried out according to the procedure described by Mehrez and Ørskov (1977). About 5 g of ground samples milled with 2 mm sieve were weighed into the nylon bags with 50 µ pore size and incubated in the rumen of fistulated bull for 1, 3, 6, 12, 24, 48 and 72 h. The animal and diet condition used in this experiment were same with the *in vitro* experiment. After incubation period, the nylon bags were thoroughly washed with cold, running water until it ran clear and dried at 60 ˚C for 48 h. The nutrient losses for each incubation time were determined. The degradation kinetics of nutrients was fitted to the exponential equation of Ørskov and McDonald (1979):

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

Where:
Y: disappearance of nutrient in rumen at time t.
a: rapidly degradable fraction.
b: slowly degradable fraction.
c: constant rate of degradation for slowly degradable fraction.

The effective degradability (Edg) of nutrients was calculated applying the equation of Ørskov and McDonald (1979):

$$Edg = a + (bc/(c+k))$$

Where:
k: rumen outflow rate of 2% per h which is at the maintenance level.

The synchronization of energy and protein was calculated from the organic matter and crude protein degradability and it was described as rumen degradable nitrogen (g) per kg of organic matter digested in the rumen (RDN (g)/OMDR (kg)).

Statistical analysis

Data on *in vitro* fermentation and *in situ* degradation were subjected to the standard analysis of variance using the general linear model (GLM) of SPSS (2007) for windows (version 16.0, Chicago, SPSS Inc.). Significance between individual means was identified using Duncan's multiple range tests (Steel and Torrie, 1980). Mean differences were considered at $P<0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance. The relationships between *in vitro* fermentation and *in situ* degradation parameters were obtained by simple linear regression.

RESULTS AND DISCUSSION

The gas volumes, except at 72 h incubation time, were different ($P<0.05$) among the experimental diets and the highest ($P<0.05$) gas volume was observed in $R_{65}D$ and $R_{50}D$ in most of incubation times (Table 1). The DM and OM disappearances of all diets were also different ($P<0.05$) in every incubation times (Table 1), in which the highest value ($P<0.05$) was found in $R_{50}D$ and the lowest was observed in $R_{60}D$ and $R_{65}D$. The CP disappearances in early incubation time were higher ($P<0.05$) in $R_{65}D$, however $R_{50}D$ had the greater values ($P<0.05$) in later incubation times.

The gas production from different carbohydrate fractions and some estimated parameters were different ($P<0.05$) among the experimental diets (Table 2). The lowest gas production ($P<0.05$) from quickly soluble fraction (a_{gas}), the highest gas production ($P<0.05$) from insoluble fraction (b_{gas}) and potential gas production ($(a+b)_{gas}$) were observed in $R_{50}D$.

The constant rate of fermentation (c_{gas}) was lowest in $R_{60}D$. Some estimated parameters such as ME, OMD and SCFA of $R_{70}D$ were lesser ($P<0.05$) than those of other diets. Although there were no differences ($P>0.05$) for PF among all diets, the lowest value was found in $R_{50}D$.

The *in situ* dry matter degradation kinetics and energy protein synchronization are shown in Table 3, in which the highest values ($P<0.05$) of rapidly degradable fraction (a_{is}) and constant rate of degradation (c_{is}) was observed in $R_{50}D$ and the lowest was found in $R_{60}D$. However, the slowly degradable fraction (b_{is}) were not different ($P>0.05$) among the experimental diets. Moreover, $R_{50}D$ also possessed the highest ($P<0.05$) effective dry matter degradability and the lowest ($P<0.05$) energy protein synchronization value.

The relationships between *in vitro* gas volume and *in situ* nutrient disappearances (DM, OM and CP) of experimental diets are shown in Table 4. Correlations ($P<0.05$) were observed in all regression equations which compared DM, OM, CP disappearance with *in vitro* gas volume.

The *in situ* effective dry matter degradability was correlated ($P<0.05$) with *in vitro* fermentation kinetics and some estimated parameters except a_{gas}, c_{gas}, ME and OMD (Table 5).

Table 1 *In vitro* gas volumes and *in situ* nutrient disappearances of experimental diets

Incubation times (h)	Experimental diets				SEM	Significant
	$R_{50}D$	$R_{60}D$	$R_{65}D$	$R_{70}D$		
***In vitro* gas volumes**						
1	2.10[b]	3.56[b]	6.33[a]	5.31[a]	0.54	**
3	6.12[c]	6.89[bc]	10.32[a]	8.88[a]b	0.56	**
6	11.99[b]	11.44[b]	15.69[a]	13.72[ab]	0.58	**
12	21.69[b]	19.16[b]	24.49[a]	21.77[b]	0.67	**
24	35.01[a]	30.27[b]	36.39[a]	32.95[ab]	0.83	**
48	47.75[a]	41.95[b]	47.45[a]	43.90[ab]	0.93	*
72	52.33	46.75	51.25	47.97	0.92	NS
***In situ* DM disappearances**						
1	25.40[a]	20.22[c]	20.76[bc]	21.84[b]	0.63	***
3	27.88[a]	21.65[c]	22.11[c]	23.69[b]	0.75	***
6	31.44[a]	23.75[c]	24.11[c]	26.38[b]	0.94	***
12	38.03[a]	27.84[c]	28.00[c]	31.45[b]	1.26	***
24	49.31[a]	35.59[c]	35.43[c]	40.41[b]	1.73	***
48	65.90[a]	49.59[c]	49.11[c]	54.53[b]	2.06	***
72	76.85[a]	62.06[b]	61.73[b]	64.73[b]	1.97	***
***In situ* OM disappearances**						
1	23.95[a]	17.26[c]	18.60[bc]	21.04[ab]	0.88	**
3	26.68[a]	18.99[c]	20.10[bc]	23.09[b]	0.98	**
6	30.59[a]	21.54[c]	22.29[c]	26.04[b]	1.15	***
12	37.76[a]	26.43[c]	26.56[c]	31.53[b]	1.45	***
24	49.89[a]	32.15[c]	34.64[c]	41.06[b]	2.19	***
48	67.26[a]	51.09[c]	49.17[c]	55.47[b]	2.14	***
72	78.30[a]	64.13[b]	61.97[b]	65.38[b]	2.04	***
***In situ* CP disappearances**						
1	30.74[b]	29.83[b]	37.17[a]	32.49[b]	0.92	***
3	33.05[bc]	31.38[c]	38.20[a]	34.40[b]	0.81	***
6	36.36[b]	33.62[c]	39.73[a]	37.12[b]	0.69	***
12	42.45[a]	37.87[b]	42.70[a]	42.06[a]	0.62	***
24	52.71[a]	45.52[c]	48.34[b]	50.28[b]	0.84	***
48	67.42[a]	57.92[c]	58.56[c]	61.85[b]	1.19	***
72	76.81[a]	67.25[b]	67.61[b]	69.21[b]	1.29	**

$R_{50}D$: roughage 50% diet; $R_{60}D$: roughage 60% diet; $R_{65}D$: roughage 65% diet; $R_{70}D$: roughage 70% diet; h: hour; DM: dry matter; OM: organic matter and CP: crude protein.
SEM: standard error of the means.
* (P<0.05); ** (P<0.01) and *** (P<0.001).
NS: non significance.

Table 2 *In vitro* fermentation kinetics and some estimated parameters of experimental diets

Parameters	Experimental diets				SEM	Significant
	$R_{50}D$	$R_{60}D$	$R_{65}D$	$R_{70}D$		
a_{gas}	1.62[b]	1.80[b]	4.19[a]	3.41[a]	0.350	***
b_{gas}	55.4[a]	48.3[b]	49.1[b]	47.0[b]	1.160	**
c_{gas}	0.0428[a]	0.0371[b]	0.0446[a]	0.0414[a]	0.001	**
$(a+b)_{gas}$	57.1[a]	50.1[b]	53.3[ab]	50.4[b]	1.050	*
Some estimated parameters						
ME (MJ/kg DM)	8.07[a]	7.94[a]	7.90[ab]	7.61[b]	0.06	*
OMD (%)	54.9[a]	54.4[a]	54.0[ab]	52.1[b]	0.41	*
SCFA (mmol/200 mg DM)	0.85[a]	0.77[b]	0.78[b]	0.73[b]	0.01	**
PF	2.92	3.64	3.29	3.45	0.11	NS

$R_{50}D$: roughage 50% diet; $R_{60}D$: roughage 60% diet; $R_{65}D$: roughage 65% diet; $R_{70}D$: roughage 70% diet; a_{gas}: gas production (mL) from quickly soluble fraction; b_{gas}: gas production (mL) from insoluble fraction; c_{gas}: gas production rate; $(a+b)_{gas}$: potential gas production; ME: metabolizable energy; OMD: organic matter digestibility; SCFA: short chain fatty acid and PF: partitioning factor for microbial protein synthesis efficiency (true digestible OM (mg)/mL of gas).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
* (P<0.05); ** (P<0.01) and *** (P<0.001).
NS: non significance.

Among the correlations, the greater percentages of coefficient of determination (R^2) were observed in regression equations that included two or more *in vitro* fermentation parameters.

Table 3 *In situ* dry matter degradation kinetics of experimental diets

Degradation kinetics	Experimental diets				SEM	Significant
	$R_{50}D$	$R_{60}D$	$R_{65}D$	$R_{70}D$		
a_{is}	24.13[a]	19.34[c]	19.74[c]	20.89[b]	0.59	**
b_{is}	74.12	80.67	80.27	71.16	1.77	NS
c_{is}	0.0174[a]	0.0097[c]	0.0099[c]	0.0136[b]	0.001	**
EDMdg	58.47[a]	46.05[c]	45.95[c]	49.33[b]	1.56	**
EPSync	21.31[d]	37.37[a]	34.95[b]	33.18[c]	1.87	***

$R_{50}D$: roughage 50% diet; $R_{60}D$: roughage 60% diet; $R_{65}D$: roughage 65% diet; $R_{70}D$: roughage 70% diet; a_{is}: rapidly degradable fraction of dry matter; b_{is}: slowly degradable fraction of dry matter; c_{is}: rate of dry matter degradation; EDMdg: effective dry matter degradability and EPSync: energy protein synchronization.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
* (P<0.05); ** (P<0.01) and *** (P<0.001).
NS: non significance.

Table 4 Relationships between *in vitro* gas production and *in situ* nutrient disappearances

Equations	Statistical criteion[1]		Significant
	R^2	RSD	
DM_{disT}= 15.233 + 0.9003 GvT	85.89	6.497	***
OM_{disT}= 13.1 + 0.9612 GvT	84.86	6.895	***
CP_{disT}= 26.849 + 0.7831 GvT	92.12	3.889	***

[1] R^2: coefficient of determination and RSD: residual standard deviation.
DM_{disT}: dry matter disappearance at time "t"; OM_{disT}: organic matter disappearance at time "t"; CP_{disT}: crude protein disappearance at time "t" and GvT: gas volume at time "t".
*** (P<0.001).

The synchronization of energy and protein was also correlated (P<0.05) with most of *in vitro* fermentation parameters (Table 6), however some parameter such as a_{gas}, c_{gas}, ME and OMD were not correlated (P>0.05) with synchronization. Correlations (P<0.001) with greater value of coefficient of determination (R^2) were found in some relationships assembled by two or more *in vitro* fermentation parameters.

The fermentation kinetics of feeds could be affected by carbohydrates fraction (Deaville and Givens, 2001) and the gas production of feed in buffered rumen fluid was also associated with feed fermentation and carbohydrate fraction (Sallam *et al.* 2008). The $R_{50}D$ possessed the lesser gas volume form quickly soluble fraction (a_{gas}), greater gas volume from insoluble fraction (b_{gas}) and fermentation rate (c_{gas}), thereby increasing gas production in later incubation times and potential gas production. Conversely, the lesser values were observed in $R_{60}D$.

The gas production from different class of feeds incubated in *in vitro* buffered rumen fluid was closely related to the production of SCFA which was based on carbohydrate fermentation (Blümmel *et al.* 1990). Therefore, $R_{50}D$ had the greater value of SCFA, an indication of energy availability to the animal. The *in vitro* gas production method has been widely used to evaluate the energy value of several classes of feeds (Getachew *et al.* 1999; Getachew *et al.* 2002). The ME and OMD values of $R_{50}D$ and $R_{60}D$ were greater than those of $R_{65}D$ and $R_{70}D$. It might be due to the different level of starch, the major carbohydrate of feeds, which were fermented by amylolytic bacteria and protozoa (Kotarski *et al.* 1992).

The PF values were not different; however they were agreed with the theoretical range for PF (from 2.74 to 4.41). The difference in *in vitro* PF values reflected to *in vivo* microbial protein synthesis, dry matter intake and methane gas production in ruminants (Blümmel *et al.* 1999).

Many researchers reported that the degradability of feedstuffs was influenced by the type of carbohydrate (soluble and insoluble carbohydrate).

The greater dry matter effective degradability could be achieved by the feedstuffs or feed contained lesser NDF content and greater degradable fraction (Maghsoud *et al.* 2008).

The greater values of rapidly degradable fraction (a_{is}) and constant rate of degradation (c_{is}) were observed in $R_{50}D$, thereby increasing dry matter disappearance and effective degradability.

The synchronization is well associated with the types of carbohydrate (soluble and insoluble) which are degraded in the rumen and amount of proteins (ruminal degradable and undegradable protein) entering the rumen. The optimum synchronization achieved by energy and ruminal nitrogen supplementation has a positive effect on microbial protein synthesis (Lardy *et al.* 2004; Elseed, 2005). In $R_{50}D$, greater in energy supply (rapidly degradable fraction, ME and SCFA) and lesser ruminal nitrogen level (PF, ruminal degradable protein-data not shown) were observed, which resulted the lesser synchronization.

The regression equations showing the relationship between *in vitro* gas volume and *in situ* disappearance of DM, OM and CP are given in Table 5, in which significant correlations were observed among those parameters.

Table 5 Estimation of dry matter degradability from *in vitro* fermentation parameters

Equations	Statistical criterion[1]		Significant
	R^2	RSD	
EDMdg= 55.574 - 2.0428a_{gas}	0.211	4.790	NS
EDMdg= 1.1646 + 0.9768b_{gas}	0.526	3.715	**
EDMdg= -0.5135 + 0.9576$(a+b)_{gas}$	0.416	4.121	*
EDMdg= 31.519 + 444.48c_{gas}	0.070	5.201	NS
EDMdg= 8.429 - 0.769a_{gas} + 0.874b_{gas}	0.550	3.619	*
EDMdg= 7.850 - 3.936a_{gas} + 1276.623c_{gas}	0.609	3.374	*
EDMdg= -5.839 + 0.943b_{gas} + 209.431c_{gas}	0.541	3.656	*
EDMdg= -3.395 - 2.668a_{gas} + 0.476b_{gas} + 889.804c_{gas}	0.673	3.085	*
EDMdg= -17.169 + 8.519$_{ME}$	0.126	5.043	NS
EDMdg= -9.040 + 1.095$_{OMD}$	0.084	5.162	NS
EDMdg= -5.639 + 71.345$_{SCFA}$	0.456	3.977	*
EDMdg= 79.270 - 8.814$_{PF}$	0.384	4.233	*
EDMdg= 43.461 + 4.210$_{ME}$ - 8.020$_{PF}$	0.412	4.136	NS
EDMdg= 49.127 + 0.528$_{OMD}$ - 8.301$_{PF}$	0.403	4.170	NS
EDMdg= 27.309 + 50.639$_{SCFA}$ - 5.055$_{PF}$	0.544	3.641	*
EDMdg= 108.761 + 212.448$_{ME}$ - 32.171$_{OMD}$ + 0.011$_{PF}$	0.665	3.122	*
EDMdg= 164.852 - 5.123$_{OMD}$ + 201.626$_{SCFA}$ + 1.182$_{PF}$	0.844	2.132	**
EDMdg= 173.076 - 39.080$_{ME}$ + 232.542$_{SCFA}$ + 1.083$_{PF}$	0.862	2.002	**
EDMdg= 200.846 - 326.514$_{ME}$ + 38.899$_{OMD}$ + 424.021$_{SCFA}$ - 1.133$_{PF}$	0.927	1.457	***

[1] R^2: coefficient of determination and RSD: residual standard deviation.
EDMdg: effective dry matter degradability; a_{gas}: gas production (mL) from quickly soluble fraction; b_{gas}: gas production (mL) from insoluble fraction; c_{gas}: gas production rate; $(a+b)_{gas}$: potential gas production; ME: metabolizable energy; OMD: organic matter digestibility; SCFA: short chain fatty acid and PF: partitioning factor for microbial protein synthesis efficiency (true digestible OM (mg)/Ml of gas).
* (P<0.05); ** (P<0.01) and *** (P<0.001).
NS: non significance.

Table 6 Estimation of energy protein synchronization from *in vitro* fermentation parameters

Equations	Statistical criterion[1]		Significant
	R^2	RSD	
EPSync= 25.519 + 2.2458a_{gas}	0.178	5.865	NS
EPSync= 92.91 - 1.2254b_{gas}	0.575	4.213	**
EPSync= 96.895 - 1.2367$(a+b)_{gas}$	0.483	4.650	*
EPSync= 61.354 - 714.977c_{gas}	0.126	6.046	NS
EPSync= 87.527 + 0.572a_{gas} - 1.149b_{gas}	0.585	4.167	*
EPSync= 90.324 + 4.818a_{gas} - 1733.531c_{gas}	0.687	3.616	**
EPSync= 107.193 - 1.157b_{gas} - 426.571c_{gas}	0.619	3.993	*
EPSync= 104.214 + 3.252a_{gas} - 0.588b_{gas} - 1255.747c_{gas}	0.755	3.198	**
EPSync= 119.567 - 11.151$_{ME}$	0.150	5.963	NS
EPSync= 108.543 - 1.427$_{OMD}$	0.099	6.138	NS
EPSync= 103.148 - 91.690$_{SCFA}$	0.524	4.462	**
EPSync= -6.195 + 11.393$_{PF}$	0.447	4.812	*
EPSync= 41.415 - 5.597$_{ME}$ + 10.338$_{PF}$	0.480	4.662	NS
EPSync= 33.432 - 0.694$_{OMD}$ + 10.719$_{PF}$	0.468	4.716	NS
EPSync= 60.187 - 64.692$_{SCFA}$ + 6.591$_{PF}$	0.628	3.945	*
EPSync= -49.687 - 296.116$_{ME}$ + 44.884$_{OMD}$ - 0.866$_{PF}$	0.823	2.720	**
EPSync= -112.501 + 6.432$_{OMD}$ - 254.258$_{SCFA}$ - 1.240$_{PF}$	0.956	1.350	***
EPSync= -119.913 + 48.284$_{ME}$ - 289.438$_{SCFA}$ - 0.992$_{PF}$	0.966	1.199	***
EPSync= -134.725 + 201.602$_{ME}$ - 20.749$_{OMD}$ - 391.573$_{SCFA}$ + 0.190$_{PF}$	0.978	0.949	***

[1] R^2: coefficient of determination and RSD: residual standard deviation.
EPSync: energy protein synchronization; a_{gas}: gas production (mL) from quickly soluble fraction; b_{gas}: gas production (mL) from insoluble fraction; c_{gas}: gas production rate; $(a+b)_{gas}$: potential gas production; ME: metabolizable energy; OMD: organic matter digestibility; SCFA: short chain fatty acid; PF: partitioning factor for microbial protein synthesis efficiency (true digestible OM (mg)/mL of gas).
* (P<0.05); ** (P<0.01) and *** (P<0.001).
NS: non significance.

This finding is consistent with the reports of researchers (Blümmel and Ørskov, 1993; Khazaal *et al*. 1993; Kamalak *et al*. 2005; Ozkan and Sahin, 2006; Sileshi *et al*. 1996) who observed the significant correlations between DM disappearance and *in vitro* gas production.

In situ effective dry matter degradability (EDMdg) of roughage based diets was highly related to the gas production (b_{gas}) from insoluble fraction and potential gas production, $(a+b)_{gas}$. These finding were in agreement with the reports of scientists (Blümmel and Ørskov, 1993; Khazaal

et al. 1993; Sileshi *et al*. 1996) who found a significant correlation between effective dry matter degradability and potential gas production. The inclusion of other parameters such as a_{gas} and c_{gas} to regression equation increased the percentage of variation of EDMdg expressed by gas production parameters. This result was also consistent in the reports (Kamalak *et al*. 2005; Ozkan and Sahin, 2006) which described that the greater correlation could be achieved with regression equations included a_{gas}, b_{gas}, c_{gas} and $(a+b)_{gas}$. However, there were no explanation about the relationship between EDMdg and other estimated parameters such as SCFA, ME, OMD and PF. In this study, SCFA and PF were significantly correlated with EDMdg, however no significance was found with ME and OMD. The SCFA and PF explained 84.4%, 86.2% and 92.7% of variation of EDMdg when it was combined with OMD, ME and (ME+OMD), respectively. Therefore, it was noted that the more inclusion of parameters in regression equations, the greater accuracy and variation of EDMdg could be achieved. For the synchronization of energy and protein, it was significantly correlated with the gas production (b_{gas}) from insoluble fraction and potential gas production, $(a+b)_{gas}$, but there were no significant relation to gas production (a_{gas}) from quickly soluble fraction and fermentation rate (c_{gas}). When two or more parameters were used in regression equations, the variation of synchronization was significantly increased from 58.5% to 75.5%. Moreover, the significant correlations were found in relationship with SCFA and PF, but not with ME and OMD alone. The SCFA and PF explained from 95.6% to 97.8% of variation of synchronization when ME, OMD and (ME+OMD) were added to the regression equation. It might be due to proper combination of parameters which are the main sources for energy and protein.

CONCLUSION

The correlations between the results of both *in situ* and *in vitro* techniques seem to be sufficiently strong to predict *in situ* disappearances of DM, OM and CP from cumulative gas volumes. Although effective dry matter degradability and energy protein synchronization were significantly correlated with *in vitro* fermentation kinetics (b_{gas} and $(a+b)_{gas}$) and some estimated parameters (SCFA and PF), the greater accuracy and variation of those parameters could be achieved by inclusion of more parameters in regression equation. Moreover, estimated parameters such as SCFA, ME, OMD and PF can give the greater percentage of correlation in compare with fermentation kinetics. It was concluded that *in vitro* gas production technique has the potential to predict the effective dry matter degradability and energy protein synchronization of roughage based diets.

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