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USAGE OF CONTINUOUS PRESSURE READER IN *IN VITRO* GAS PRODUCTION TECHNIQUE FOR EVALUATION OF FEEDSTUFFS FOR RUMINANTS

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Abstract

This experiment was conducted to determine possibility of usage of automatic system that continuously record pressure and sample gas at certain intervals in in vitro gas production technique. Rumen fluid was collected from 2 ruminally cannulated Holstein heifers weighing an average of 400 kg. Medium was prepared by mixing macromineral (200 ml), micromineral (0.1 ml), buffer (200 ml), reduction (40 ml) and resazurin (1 ml) solutions as well as distilled water (400 ml). The mixtures contained alfalfa hay, maize silage, wheat, maize, cottonseed meal, and soybean meal. Mixtures differing in the roughage:concentrate ratio (20:80, 40:60, 60:40, and 80:20) were formulated to contain rapidly fermentable fraction (B1+B2), insoluble but slowly fermentable fraction (B3,) and 50% of these fractions at three different fermentation characteristics. Gas productions at 6, 12, 24, and 48 h relative to incubation determined using system recording continuously. In automatic system, "dual-pool logistic equation" representing factions either soluble in NDF solution and rapidly fermentable fraction (NDF-F) and fractions or insoluble in NDF solution, but slowly fermentable (NDF-S) were used. The fermentation was completed within 12 h in fully automatic system. While cumulative gas production from NDF-F was positively correlated with gas production within 3 h (r=0.77, P<0.001), cumulative gas production from NDF-S was positively correlated with gas production within 3-20 h (r=0.88, P<0.001). Differences in time to reach maximal fermentation rate from NDF-F and NDF-S increased with increasing NDF (r=0.76, P<0.001) and ADF (r=0.85, P<0.001) levels. These data suggest that fermentation kinetics parameters, such as lag time and time to reach maximal fermentation rate from fractions soluble and insoluble in NDF solution, should be considered in formulation and evaluation of rations.

Keywords: *automatic gas system, in vitro gas production technique, feedstuff evaluation.*

INTRODUCTION

In vitro gas production technique is considered a unique method for determining nutritive value of feedstuffs and compound feeds (Getachew et al., 2005). This technique is employed ration evaluation when excess carbohydrates are fed to minimize metabolic disturbances. Projection of fermentation characteristics and kinetics can help elucidate intake depression especially in early lactation (Johnston and Tricarico, 2007; Pell and Schofield, 1993). Using systems that record continuously allow determination of kinetics of gas, which enables to reduce production of CH_4 and (Ramin and Huntanen, 2012). This system provides information that is invaluable to project the fate of nutrients and establish alternative feeding management. Automated *in vitro* gas system time-dependent changes are modeled to determiner fast and slow degradable fractions (Johnston and Tricarico, 2007; Pell and Schofield, 1993; Schofield et al., 1994) or degradable and undegradable fractions as well as gas production (Dijkstra et al., 2005; France et al., 2005). Degradation of fractions (B_1- rapidly degradable fraction, mainly starch; B_2- slowly degradable soluble fraction; and B_3- insoluble but slowly degradable fraction, mainly cellulose and hemicellulose) is described in detail. Imbalance between rapidly and slowly degradable fractions leads to low production and dry matter intake, acidosis, laminitis, milk fat depression, displaced abomasum, and liver abscess (Johnston and Tricarico, 2007).

MATERIALS AND METHODS

Rumen fluid was obtained from two ruminally cannulated Holstein heifers. They were fed twice daily. Ration consisted of the roughage:concentrate ratio of 60:40 to meet maintenance plus 0.5 kg weight gain and contained 5 kg alfalfa (13.43% CP and 43.50% NDF) and 3.5 kg compound feed (17.32% CP and 37.82% NDF).

The mixtures included alfalfa hay, corn silage, wheat grain, corn grain, cottonseed meal, and soybean meal. Corn silage, wheat grain, and soybean meal predominantly contain rapidly fermentable fractions (F), others predominantly contain insoluble, but slowly fermentable fractions (S) (B_3) (Mahanna, 2010). The mixtures were prepared to exist in different roughage:concentrate ratios (20:80, 40:60, 60:40, and 80:20). Final mixtures were predominant in F and S as well as equal amount of them (50F). They were isonitrogenous.

To determine gas production and gas kinetics NDF residues of these two pools (NDF-F and NDF-S) were obtained (Van Soest et al., 1991; Pell and Schofield, 1993; Schofield and Pell, 1995). Ground mixtures and their NDF residues (460 mg) were put in 100-ml Pyrex tubes containing ruminal fluid medium [(macromineral (200 ml), micromineral (0.1 ml), buffer (200 ml), reduction (40 ml) and resazurin (1 ml) solutions as well as distilled water (400 ml)] and incubated at 39°C (Menke and Steingass, 1988). Pressure due to gas production was monitored and recorded every minute using data-logger (RHT50, Extech Instruments, USA). Gas was released 6 times within 12 h, 3 times within 12-24, and 3 times within 24-48 h. Cumulative gas production was calculated using pressure recorded by digital manometer (Lopez et al., 2007).

"dual-pool logistic equation" (soluble in NDF solution and rapidly fermentable; insoluble in NDF soluble and slowly fermentable) was used to calculate gas kinetics in curve subtraction technique (Schofield et al., 1994; Schofield and Pell, 1995). Formulas were:

$$\text{Gas, ml} = V_{1F}\,\{1+\exp(2+4S_1(\lambda_1-t))\}^{-1} + V_{2F}\,\{1+\exp(2+4S_1(\lambda_2-t))\}^{-1}$$

V_{1F} and V_{2F}: maximal gas in both pools.

S_1 and S_2: specific fermentation constant for both pools.

t: incubation time.

λ: lag time (λ_1 and λ_2 represent pools)

Data were subjected to 2-way ANOVA in a completely randomized design in which groups were arranged in 4 x 3 factorial fashion (SPSS, 2006). The linear model in data analyses was as follows:

$$Y_{ijk} = \mu + (R{:}C)_i + (FC)_j + (R{:}C \times FC)_{ij} + e_{ijl}$$

Y_{ijk} = response variable
μ = population mean
$R{:}C_i$ = i^{th} roughage:concentrate ratio
FC_j = j^{th} fermentation characteristics
e_{ijk}= experimental error

RESULTS AND DISCUSSIONS

Fermentation was almost completed within 12 h and was at a very low level between 24 and 48 h. As the R:C ratio decreased, pH decreased linearly (P<0.023). The substrate rich in F decreased pH more dramatically than the substrate rich in S (P<0.002). As the incubation advanced NH_3-N concentration increased (P< 0.002). Lag time for fractions soluble in NDF solution there was no effect of the roughage proportion and fermentation characteristics (F and S). However, lag time for fractions insoluble in NDF solution increased with increasing the roughage proportion (P<0.025) and providing S (P<0.001). The gas production decreased from NDF-F (P<0.053) and increased from NDF-S (P<0.005) as the roughage proportion increased. Expectedly, gas production from NDF-S was low at earlier phase of incubation. Gas production from NDF-F and NDF-S was similar 24 h after incubation.

There was a positive correlation between cumulative gas production and gas production within 3 h of incubation from NDF-F (r = 0.77; P<0.001). For NDF-S, this relationship was evident cumulative gas production and gas production between 3 and 20 h of incubation (r = 0.88; P<0.001).

Difference in times to reach maximal fermentation of substrates soluble and insoluble in NFD got longer as the NDF level increased (r =0.76; P<0.001). Similar observation was noted for ADF, especially for fractions of S (r = 0.85; P<0.001) and difference in times to reach maximal fermentation for F and S (r = 0.85; P< 0.001). These indicated importance of focusing on times to reach maximal fermentation of F and S substrates in ration evaluation. The specific ratio of NDF-S for concentrates was greater than that for roughages (Table 1).

Table 1. Gas production parameters of substrates differing in roughage:concentrate (R:C) ratio and fermentation characteristics (FC; F- fast; S- Slow; 50F- mixture of F and S) in fully automated *in vitro* gas production system

		Gas Measurements[1]									
R:C	FC	CGP (ml)	S-NDF (ml)	Is-NDF (ml)	cS-NDF (h^{-1})	cIs-NDF (h^{-1})	tS-NDF (h)	tIs-NDF (h)	Δt (h)	S-Lag (h)	Is-Lag (h)
	F	163	135	27	0.087	0.105	7.5	6.3	-1.2	1.68	1.48
20:80	50F	152	112	40	0.078	0.095	7.4	7.2	-0.2	1.12	2.03
	S	142	107	35	0.082	0.090	7.3	9.6	2.3	1.00	3.94
	F	165	129	37	0.087	0.098	6.8	7.0	0.2	1.03	1.93
40:60	50F	151	112	40	0.081	0.091	6.3	8.4	2.1	0.28	2.97
	S	145	109	36	0.087	0.085	6.9	10.9	4.0	1.15	4.95
	F	141	96	45	0.087	0.080	7.3	7.1	-0.1	1.41	0.85
60:40	50F	141	97	44	0.114	0.071	6.2	9.3	3.2	1.73	2.35
	S	143	111	32	0.103	0.078	5.6	12.2	6.5	0.83	5.74
	F	155	99	56	0.072	0.072	7.5	8.5	1.0	0.60	1.06
80:20	50F	152	105	47	0.093	0.066	4.8	11.5	6.8	0.70	4.08
	S	142	108	33	0.110	0.080	5.5	13.6	8.2	0.98	7.21
SEM		2.36	2.66	1.28	0.03	0.02	0.15	0.30	0.39	0.14	0.28
Effect		-- P < --									
R:C		0.287	0.053	0.005	0.051	0.001	0.001	0.001	0.001	0.437	0.025
FC		0.107	0.385	0.005	0.123	0.118	0.001	0.001	0.001	0.812	0.001
R:CxFC		0.214	0.006	0.843	0.085	0.369	0.022	0.273	0.001	0.639	0.098

[1]CGP = cumulative gas production. S-NDF = gas produced from fraction soluble in NDF solution. Is-NDF = gas produced from fraction insoluble in NDF solution. cS-NDF = maximal gas production constant for fraction soluble in NDF solution. cIs-NDF = maximal gas production constant for fraction insoluble in NDF solution. tS-NDF = time occurring maximal gas production from fraction soluble in NDF solution. tS-NDF = time occurring maximal gas production from fraction insoluble in NDF solution. Δt = difference in times to reach maximal fermentation from fractions soluble and insoluble in NDF solution. S-Lag = lag time for fractions soluble in NDF solution. Is-Lag = lag time for fractions insoluble in NDF solution.

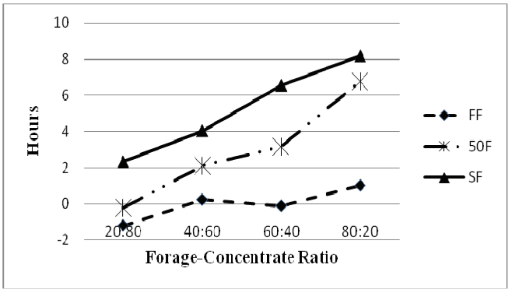

Figure 1. Times to reach maximal gas production from fractions soluble (NDF-F) and insoluble (NDF-S) in NDF solution using fully automatic *in vitro* gas system (SEM = 0.39). FF = fractions rapidly fermented; SF = fractions slowly fermented; 50F = mixture of FF and SF.

Microbial mass started to differ 12 h after incubation depending upon fermentation characteristics of substrates. It was greater for FF than for SF. Microbial mass production efficiency was 43.3, 44.0, 45.4, and 46.0% for 20, 40, 60, and 80% the roughage proportions, respectively at the end of 24-h incubation (P<0.001).

CONCLUSIONS

Lag time and time to reach maximal fermentation are important fermentation kinetics parameters and differ by solubility of fractions in NDF solution. Considering these parameters could improve nutritional efficiency and well-being of the ruminant animal.

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