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Degradation Kinetics of Carbohydrate Fractions of Ruminant Feeds Using Automated Gas Production Technique

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ABSTRACT : The current ruminant feeding models require parameterization of the digestion kinetics of carbohydrate fractions in feed ingredients to estimate the supply of nutrients from a ration. Using an automated gas production technique, statistically well-defined digestion rate of carbohydrate, including soluble carbohydrate, can be estimated in a relatively easy way. In this study, the gas production during *in vitro* fermentation was measured and recorded by an automated gas production system to investigate degradation kinetics of carbohydrate fractions of a wide range of ruminant feeds: corn silage, rice straw, corn, soybean hull, soybean meal, and cell mass from lysine production (CMLP). The gas production from un-fractionated, ethanol insoluble residue and neutral detergent insoluble residue of the feed samples were obtained. The gas profiles of carbohydrate fractions on the basis of the carbohydrate scheme of the Cornell Net Carbohydrate and Protein System (A, B1, B2, B3 and C) were generated using a subtraction approach. After the gas profiles were plotted with time, a curve was fitted with a single-pool exponential equation with a discrete lag to obtain kinetic parameters that can be used as inputs for modern nutritional models. The fractional degradation rate constants (Kd) of corn silage were 11.6, 25.7, 14.8 and 0.8%/h for un-fractioned, A, B1 and B2 fractions, respectively. The values were statistically well estimated, assessed by high t-value (>12.9). The Kd of carbohydrate fractions in rice straw were 4.8, 21.1, 5.7 and 0.5%/h for un-fractioned, A, B1 and B2 fractions, respectively. Although the Kd of B2 fraction was poorly defined with a t-value of 4.4, the Kd of the other fractions showed t-values higher than 21.9. The un-fractioned corn showed the highest Kd (18.2%/h) among the feeds tested, and the Kd of A plus B1 fraction was 18.7%/h. Soybean hull had a Kd of 6.0, 29.0, 3.8 and 13.8%/h for un-fractioned, A, B1 and B2, respectively. The large Kd of fraction B2 indicated that NDF in soybean hull was easily degradable. The t-values were higher than 20 except for the B1 fraction (5.7). The estimated Kd of soybean meal was 9.6, 24.3, 5.0%/h for un-fractioned, A and B1 fractions, respectively. A small amount of gas (5.6 ml at 48 ho of incubation) was produced from fermentation of CMLP which contained little carbohydrate. In summary, the automated gas production system was satisfactory for the estimation of well defined (t-value >12) kinetic parameters and Kd of soluble carbohydrate fractions of various feedstuffs that supply mainly carbohydrate. The subtraction approach, however, should be applied with caution for some concentrates, especially those which contain a high level of crude protein since nitrogen-containing compounds can interfere with gas production. (**Key Words :** Gas Production Technique, Fractional Rate of Carbohydrate Digestion, Digestion Kinetics of Soluble Carbohydrate)

INTRODUCTION

The ability of rumen microbes to digest structural carbohydrate (SC) determines the energy metabolism of ruminant animals (Schofield, 2000). The non-structural carbohydrate (NSC) and SC are degraded and taken up by the microbes in the rumen and converted into forms such as volatile fatty acids which can be utilized by the host animal. This is a time-dependent dynamic process, which means there is competition between digestion and passage of carbohydrate in the rumen. In this regard, the measurement and interpretation of carbohydrate digestion rate and digestibility should be carefully examined in a study of ruminant nutrition (Schofield, 2000).

Classical *in vitro* residual techniques (e.g. the Tilley and Terry (1963) method) have been widely used to estimate kinetic parameters of digestion, which is commonly expressed as fractional rate of digestion (Kd). The residual techniques measure the disappearance of nutrients using residue analysis after filtration, and are easily applicable.

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However, they are labor- and time-intensive, and thus it is hard to obtain enough measurements for understanding the time-dependent degradation characteristic of carbohydrates. On the contrary, the gas production technique measures the appearance of products (Menke and Steingass, 1988). This has an advantage in that the system can be easily automated by using a pressure sensor, analog to digital card, and a personal computer to measure and record the gas produced. The automated gas production system developed by Pell and Schofield (1993) measures the pressure change of a closed bottle during *in vitro* incubation. At more than 200 time-points over 48 h of incubation, the pressure inside a culture bottle is automatically measured, and the accumulated gas is estimated based on the principles of physical chemistry. These time-series data points are enough to be fitted with non-linear regression to estimate well defined kinetic parameters. The t-value of 12 is normally used to determine whether the parameter is adequately defined (Pell et al., 2000). Another advantage of the automated gas production system is that digestion kinetics of soluble carbohydrates can be studied because it measures not degradation but production. Using the gas production system, the Kd of soluble carbohydrates can be estimated with the subtraction approach proposed by Schofield and Pell (Schofield and Pell, 1995a).

Degradation of soluble carbohydrate is commonly assumed to occur very fast (>100%/h) (Sniffen et al., 1992); however, many researchers have reported that not all the soluble nutrients are degraded as such (Mahadevan et al., 1980; Wallace and Kopecny, 1983; Schofield and Pell, 1995a; Doane et al., 1998; Chen et al., 1999). Degradation rate of soluble carbohydrates in a ruminant ration needs to be considered since it is related with microbial growth and the ruminal pH (Russell, 2002). Moreover, the current animal feeding models, such as the beef NRC level 2 (2000), the dairy NRC (2001) and Cornell Net Carbohydrate and Protein System (CNCPS, Fox et al., 2004), require parameterization of ruminal kinetics for each carbohydrate fraction to estimate degradation of carbohydrate, microbial fermentation and energy utilization by the host animal, which are eventually used in predicting the animal performance. Particularly, the current CNCPS divides soluble carbohydrate into 4 sub-fractions according to their effect on rumen fermentation (Lanzas et al., 2007). Unfortunately, few data are available that estimated Kd of soluble carbohydrate fractions of ruminant feeds that are commonly used in the field. This may result in inaccurate estimation of nutritive value of feeds when modern ration formulation models are applied in the field.

The objective of this study was to evaluate the use of the automated gas production system to estimate Kd of carbohydrate fractions of a wide range of ruminant feeds that are commonly used in Korea. The tested feeds include corn silage, rice straw, corn, soybean hull and soybean meal. Gas production profile of cell mass from lysine production (CMLP) was also measured to evaluate the impact of high nitrogen content on the gas production. Using the subtraction approach, Kd of soluble carbohydrate fractions was estimated. The values obtained in this study are expected to provide useful information for formulating a ruminant ration in the field.

MATERIALS AND METHODS

Chemical analysis

The samples were selected to represent a wide range of ruminant feeds: corn silage (grain-type forage), rice straw (forage), corn (energy concentrate), soybean hull (plant-origin byproduct), soybean meal (protein concentrate) and CMLP (bacteria-origin byproduct). Rice straw was chosen because it is the major forage in Korea (Seo, 2005) and CMLP was included because it is mainly composed of CP (72.3% DM). Nutritive value of CMLP for ruminants was recently evaluated (Seo et al., 2008a, b).

All feeds were dried at 60°C and ground in a Wiley mill (Arthur H. Thomas, Philadelphia, PA) through a 1 mm screen prior to analysis. Dry matter content of the samples used for the forward analysis was 92.0%, 94.9%, 88.9%, 90.7%, 87.9% and 86.3% for corn silage, rice straw, corn, soybean hull, soybean meal and CMLP, respectively. All samples underwent proximate analysis (AOAC, 1984), and fiber analysis as described by Van Soest et al. (1991). The permanganate lignin and ash procedures were as described by Goering and Van Soest (1970).

Preparation of ethanol insoluble residue and isolated NDF

Ethanol insoluble residue (EIR) was obtained by continuously stirring 0.5 g of the sample in 100 ml of 80% ethanol (v/v) for 4 h at room temperature. The sample was filtered through a 37 μm nylon mesh (Tetko, Briarcliff Manor, NY) and rinsed three times with 80% ethanol and once with acetone under mild vacuum (Chen et al., 1999; Hall et al., 1999). The sample was then dried at 100°C, and DM was measured. The EIR content of a feed was expressed as a percentage of DM.

The NDF used for fermentation was obtained by soaking 0.5 g of sample in 15 ml of 8 M urea plus 0.1 ml Termamyl (NOVO Biochemical Industries Inc., Franklinston, N.C.) overnight and then autoclaving the sample in 50 ml of ND solution plus 0.05 ml Termamyl at 105°C for 60 min. (Pell and Schofield, 1993). The NDF residue was then filtered through a 37-μm nylon mesh cloth (Tetko, Briarcliff Manor, NY), soaked in 1 M $(NH_4)_2SO_4$ at 39°C overnight and filtered through nylon mesh again and washed with hot water, ethanol, and acetone (Chen et al.,

Table 1. Chemical composition of the samples used in the *in vitro* fermentation

Composition (% DM)	CS	RS	Corn	SH	SBM	CMLP
Crude protein	10.0	6.3	10.4	21.7	51.3	72.3
Ether extract	2.9	1.5	4.0	3.8	2.7	5.3
Ash	5.9	12.1	1.2	8.27	6.5	5.3
NSC	36.9	11.2	73.3	30.6	28.2	14.4
NDF	45.0	70.4	11.9	36.2	13.2	2.9
ADF	26.4	50.5	3.0	10.2	10.0	1.1
Lignin	3.8	7.7	1.7	1.3	1.3	0.8
EIR	85.6	92.4	93.3	76.2	80.3	66.0

CS = Corn silage; RS = Rice straw; SH = Soybean hull; SBM = Soybean meal; CMLP = Cell mass from lysine production; NSC = Non-structural carbohydrate which was computed as described in Fox et al. (2004); NDF = Neutral detergent insoluble fiber; ADF = Acid detergent insoluble fiber; EIR = Ethanol insoluble residue.

1999). The NDF residues were dried in a 60°C oven overnight prior to subsequent DM analysis and fermentation.

In vitro gas production

Ruminal fluid was collected 2 to 3 h after the morning feeding from non-lactating Holstein cows that were fed either half concentrate (13% CP and 72% TDN) and half timothy hay or only timothy hay for fermentation of concentrates or forage, respectively. The strained ruminal fluid, obtained by filtering the ruminal fluid through eight layers of cheesecloth and glass wool was transferred into a 100 ml serum bottle and stored at 39°C under strict anaerobic conditions.

Unfractionated, EIR, and NDF residue of each sample in an amount of 100 mg were fermented *in vitro* in 50 ml serum bottles containing 2 ml of ruminal fluid and 8 ml of anaerobic medium prepared as described by Goering and Van Soest (1970). Gas production was measured using the method of Pell and Schofield (1993) with minor modifications as described by Schofield and Pell (1995b). The pH and NDF disappearance were measured at the end of fermentation.

Gas volumes, converted from voltage changes and corrected to 1 atmosphere pressure were calculated to represent production from 100 mg NDF. Gas associated with the A and B1 fractions was estimated using gas curve subtraction (Schofield and Pell, 1995a). Gas produced from the fraction A was determined by subtracting the gas yield of EIR preparation from that of the un-fractionated feed sample. Likewise, the gas volume from the soluble fiber and starch (B1 fraction) was determined by difference between the gas produced by EIR and NDF residue of the sample. When ethanol extract was negligible, the difference between the gas produced by un-fractionated and NDF residue was assumed to be the gas produced from fraction A+B1. The gas production from B2 fraction was obtained by measuring the gas production from NDF residue. Each fraction (unfractionated, EIR, and NDF residue) were fermented at the same time in the system and replicates were performed on a different day with different rumen fluid.

Curve fitting and estimation of fractional rate of carbohydrate digestion

The gas production profiles obtained during incubation were fitted to mathematical models and associated statistics were performed using the TableCurve 2D program (version 4.0; Jandel Scientific software, C.A.) in order to estimate fractional rate of gas production. Even though a model with more parameters (e.g. the two-pool logistic model) usually increases the goodness of fit, assessed by F value, most rumen models (Baldwin et al., 1988; NRC, 2001; Fox et al., 2004) assume that fermentation in the rumen follows first-order kinetics (enzyme excess, substrate limited). Thus, a one-pool simple exponential model with a discrete lag, as described by Schofield et al. (1994), was chosen to describe and fit the gas production curve. The equation was as follows:

$$V_T = 0 \qquad (0 \le T \le L)$$

$$V_T = Vmax \times (1-EXP(-K \times (T-L))) \qquad (T \ge L)$$

Where, T is time (h), L is discrete lag time (h), EXP is the exponential function, K is fractional rate of gas production (1/h), Vmax is maximum gas production (ml) after the asymptote is reached, and V_T is gas produced at time T (ml).

When the amount of gas produced was assumed to be proportional to substrate digestion (Schofield et al., 1994), the fractional rate of gas production was the same as Kd of the sample.

A problem of using the subtraction approach was that gas production was decreased after the asymptote was reached. The declining part of the curve was an artifact arising from the curve subtraction approach (Pell et al., 2000). Fractional rate was estimated assuming gas production was persistent after the asymptote was reached (Pell et al., 2000).

RESULTS AND DISCUSSION

Chemical analysis

The chemical composition of the samples is presented in Table 1. Corn silage was high in NDF (45% DM) as well as NSC (36.9% DM), while rice straw contained little NSC (11.2% DM) and a large amount of NDF (70.4% DM). A large portion of NDF in rice straw was slowly degradable possibly due to its high content of ADF (50.5% DM) and lignin (7.7% DM). Most carbohydrate of corn was NSC (73.3% DM) and mostly ethanol insoluble (Table 1). Soybean hull contained a more balanced distribution of CP (21.7% DM), NSC (30.6% DM) and NDF (36.2% DM) than other feed ingredients used in the present study. Since NDF of soybean hull had little ADF (10.2% DM) and lignin (1.3% DM), the Kd and digestibility of NDF in soybean hull were likely to be high. Soybean meal and CMLP were high in CP (Table 1). The carbohydrate fraction in soybean meal was high (41.4% DM) but, as expected, little carbohydrate was contained in CMLP (17.3% DM).

Ethanol extractable fraction contains organic acids, oligosaccharides, some ether extract, and soluble crude protein (Hall et al., 1999). Among these components, organic acids, oligosaccharides and some crude protein are fermentable, and, with the exception of protein, produce gas during fermentation (Pell, 1997). Therefore, gas production from the ethanol extractable fraction can represent ruminal digestion of carbohydrate A fraction (Pell, 1997). Chemically, organic acids are not carbohydrate and do not provide energy directly to the animal, but it is reasonable to place them in the carbohydrate fraction A for nutritional purposes (Lanzas et al., 2007). For example, fermentation of malate does not yield energy; however, malate can stimulate a ten-fold increase in the ability of *Selenomonas ruminantium* to use lactate (Callaway and Martin, 1997). Lactate can be used by the rumen bacteria yielding acetate, propionate, and CO_2 with a yield of 1 mole of ATP per 3 mole of lactate fermented (Pell, 1997). In this study, corn silage contained 14.39% of ethanol soluble DM. Most of this fraction is organic acids and particularly lactate (Pell, 1997; Van Soest, 1994). It has been reported that lactate comprises 10% of DM in silage in general (Doane et al., 1997). Rice straw and corn contained a small amount of ethanol extractable fraction, 7.6% and 6.7% DM, respectively, due to their low content of sugar. Soybean meal (19.8% DM) and soybean hull (23.8% DM) contained a relatively large amount of ethanol soluble materials. CMLP also contained a large amount of ethanol extractable fraction (34.0% DM), and most of this fraction was probably soluble nitrogen (Table 1).

In vitro ruminal fermentation and gas production

The pH after 48 hour incubation was higher than 6.2 in all culture tubes, which indicates microbial fermentation was normal (Schofield, 2000). The gas curves for corn silage, rice straw, corn, soybean hull, soybean meal, and CMLP, normalized to the amount of the fraction contained in 100 mg DM, are shown in Figure 1 to 6.

The gas production of the un-fractionated feedstuffs at 48 hours of fermentation was the highest in corn grain, followed by corn silage, soybean meal, soybean hull, and rice straw. As expected, fermentation of CMLP produced only a small amount of gas. A typically shaped gas production curve was observed for all feed samples tested in the present study, which included a lag phase at the beginning, an exponential phase, and then a plateau phase.

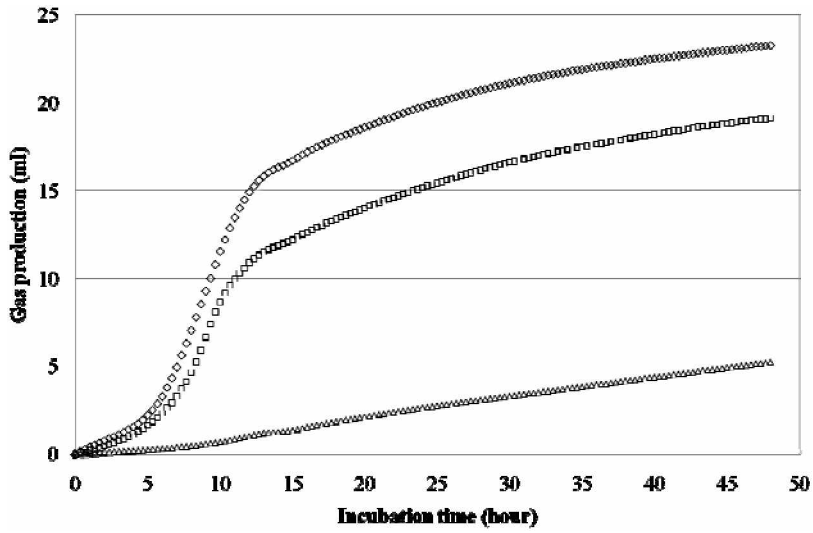

Figure 1. Gas production profile of corn silage (ml/100 mg DM). ◊, un-fractionated; □, ethanol insoluble residue; Δ, neutral detergent insoluble residue.

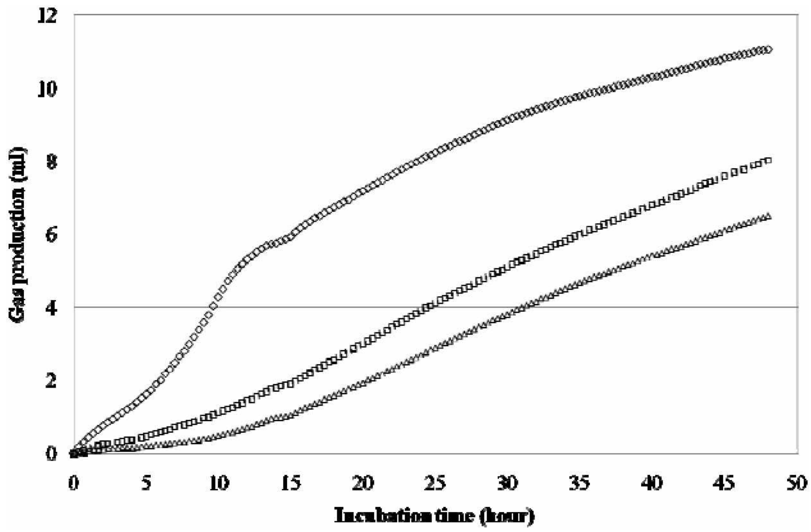

Figure 2. Gas production profile of rice straw (ml/100 mg DM). ◊, un-fractionated; □, ethanol insoluble residue; Δ, neutral detergent insoluble residue.

Rice straw, however, did not reach a plateau phase within 48 h.. Interestingly, un-fractionated soybean hull reached an asymptotic gas production between 10 and 15 h, and gas was produced at a slower rate thereafter. This may imply the existence of multiple pools in soybean hull: an easily and a slowly degradable pool. Schofield et al. (1994) suggested a dual pool logistic equation with a single discrete lag was the best model to fit and interpret gas production profiles. Although a dual pool model seems reasonable considering the heterogeneity of feedstuffs, we used a simple exponential equation with a single discrete lag to estimate Kd of each fraction because it is still commonly used and easy to be integrated with the current feeding models. Another possible explanation for the second rise in gas production from un-fractionated soybean hull is that gas was produced by fermentation of microbial cell mass which contains some polysaccharides (Schofield et al., 1994). Microbial lysis begins between 10-15 h of *in vitro* fermentation (Schofield, 2000) and further gas can be produced by the process which is not thought to happen *in vivo* with continuous flow of materials. However, the second increase in gas production did not happen when neutral detergent insoluble residue of soybean hull was fermented (Figure 5).

The gas production profiles from EIR and NDF of forage (corn silage and rice straw) were similar to those reported elsewhere (Doane et al., 1998; Pell, 1997; Schofield and Pell, 1995a). However, the gas production

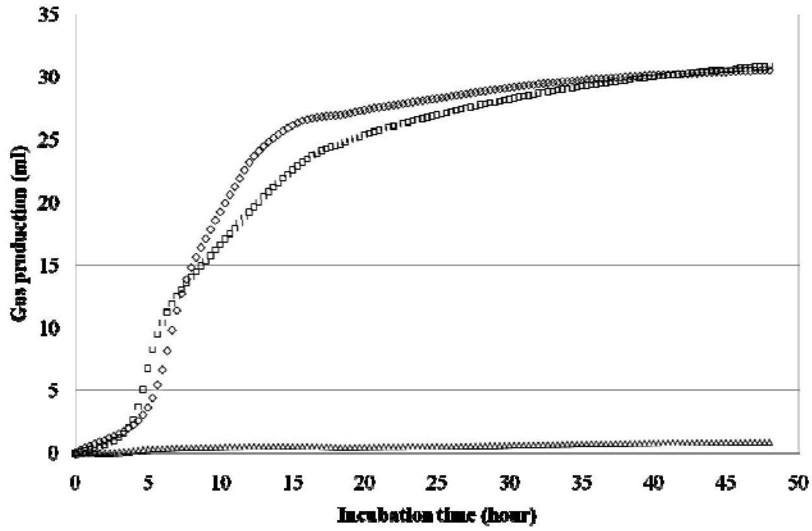

Figure 3. Gas production profile of corn (ml/100 mg DM). ◊, un-fractionated; □, ethanol insoluble residue; Δ, neutral detergent insoluble residue.

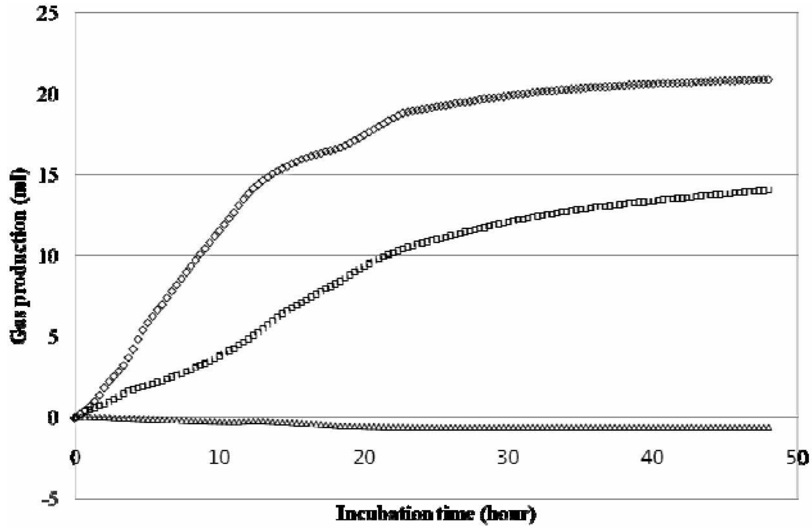

Figure 4. Gas production profile of soybean meal (ml/100 mg DM). ◊, un-fractionated; □, ethanol insoluble residue; Δ, neutral detergent insoluble residue.

from EIR of corn and soybean hull indicated that use of the subtraction method could be questionable for concentrate feeds. In the fermentation of corn, the patterns of gas production from un-fractionated and EIR were similar to those reported by Chen et al. (1999); however, the gas produced from EIR exceeded that from the un-fractionated from 3.5 to 7.5 h of incubation (Figure 3). A similar but more problematic issue was observed in the gas production from soybean hull. More gas was produced from EIR than un-fractionated after 17 h incubation (Figure 5). One possible explanation is elimination of interaction among the components in the feed during extraction. In other words, certain ethanol extractable compounds in soybean hull which inhibit microbial growth and rumen fermentation may be extracted during the ethanol extraction procedure, so more fermentation occurs in EIR. Another possibility is that the extraction procedure disrupts physical or chemical structure of soybean hull to become more fermentable by rumen microbes. The latter was not the case in corn grain (Chen, 1999). The curve subtraction technique is based on the assumption that the preparation of the forage fractions (EIR and isolated NDF) does not alter the fermentation of the insoluble carbohydrates (Schofield and Pell, 1995a). This effect could be evaluated by comparing NDF digestibility of the un-fractionated feed with that of EIR and NDF residues (Doane et al., 1998). Some researchers

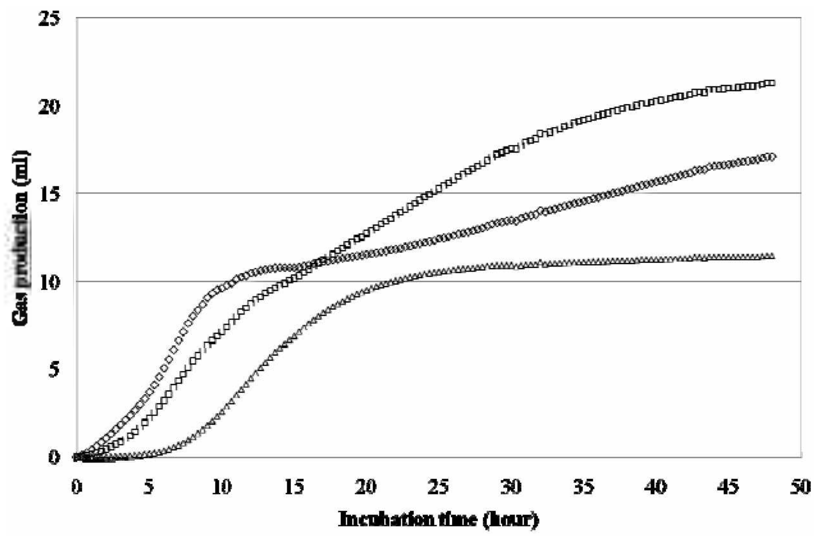

Figure 5. Gas production profile of soybean hull (ml/100 mg DM). ◊, un-fractionated; □, ethanol insoluble residue; Δ, neutral detergent insoluble residue.

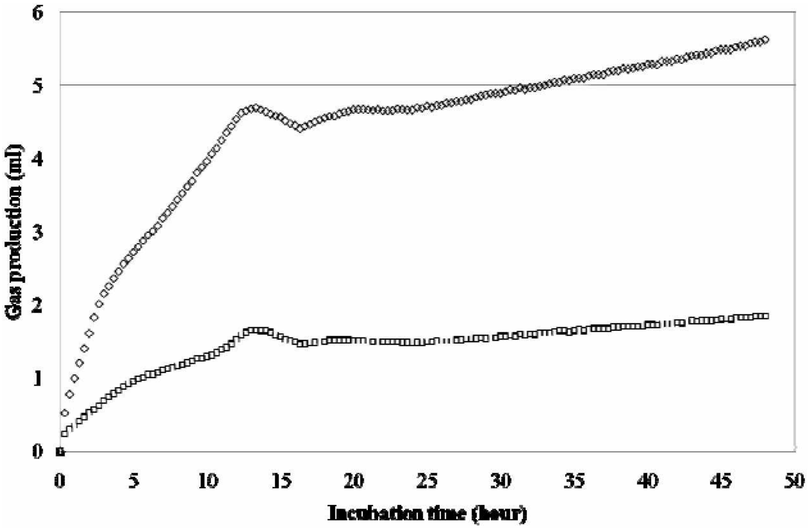

Figure 6. Gas production profile of CMLP (ml/100 mg DM). ◊, un-fractionated; □, ethanol insoluble residue.

reported that NDF digestibility of the un-fractionated and the insoluble residues in forage (Doane et al., 1998) and in corn grain (Chen et al., 1999) were similar. However, based on our data, the values were not always consistent especially for concentrates (data are not shown). Also, soluble sugars may alter the fermentation of the associated fiber components by changing both the rate and extent (Doane et al., 1998). Removal of the soluble fractions during extraction eliminates the possible interaction that may occur during the fermentation of the un-fractionated feedstuff. In spite of these two problems mentioned earlier, for now the curve subtraction technique may be considered as one of the best ways to obtain Kd of soluble carbohydrate fractions; however, development of a better method is needed for some feeds (i.g. soybean hull).

The gas produced from fermentation of neutral detergent insoluble residue of soybean meal showed negative values (Figure 4) in spite of its NDF content (13.2% DM). This should not happen because our system measures pressure changes in the closed vessel. The gas is produced in two ways, one is direct gas from fermentation and the other is indirect gas produced by reaction of volatile fatty acids with the bicarbonate (Schofield, 2000). The latter process is an acid-base reaction to maintain pH in the solution. During the *in vitro* fermentation of feeds abundant in protein such as soybean meal and CMLP, nitrogen-containing compounds can interfere with the reaction as they bind with hydrogen ion and increase pH, and thus $HCO_3^-+H^+\leftrightarrow H_2CO_3\leftrightarrow CO_2+H_2O$ reaction may occur reversibly. Likewise, fermentation of CMPL, composed mainly of crude protein (72.3% DM), produced as little as 5 ml gas for 48 h of incubation. Considering the gas yield from protein is about one-half the volume from an equivalent amount of carbohydrate (Menke and Steingass, 1988), the gas produced from CMLP was significantly lower than the theoretical value. This suggests that the gas production technique may not be applicable for feed containing a high level of nitrogen.

Fractional rate of degradation of carbohydrate fractions

To obtain fractional degradation rate constant, we followed the subtraction approach described by Schofield and Pell (1995a). This approach provides relatively easy estimates of Kd of carbohydrate fractions, although the fermentation of three separate feed fractions is still needed. The values and statistics of Kd for each fraction in the feed samples are presented in Table 2. The Kd of corn silage were 11.6, 25.7, 14.8 and 0.8%/h for un-fractionated, A, B1 and B2 fractions, respectively. The statistical t-values of all the parameters were higher than 12.9. The Kd estimates were different from those in the CNCPS feed library (Fox et al., 2003). The Kd values of typical corn silage in the feed library were 10, 25-40 and 4.0-7.0%/h for A, B1 and B2 fractions, respectively. There have been critiques that Kd values in the CNCPS feed library were higher than those expected (Alderman, 2001). CNCPS has recently changed the Kd of corn silage carbohydrate A fraction from 300%/h to 10%/h on the basis of the results from gas production measurement. CNCPS assumes that the carbohydrate A fraction in corn silage is 20% of the non-fiber carbohydrate and its Kd is 10%/h, accounting for low energy yield from organic acids (Fox et al., 2003). However, the rate for A fraction seems to be underestimated according to our data. This discrepancy might be due to the fact that chemical composition among corn silages were variable and/or the discount used in the CNCPS was not very representative. Our Kd value for the B1 fraction was similar to the value (14.2%/h) reported by Doane et al. (1998). Kd of the B2

Table 2. Fractional rate of gas production estimated by fitting gas production curves of carbohydrate fractions using the subtraction method with one-pool exponential with a single discrete lag model

Sample	Fraction	R^2	Kd (hr^{-1})	t-value	95% confidence interval	
					Min.	Max.
CS	Un-fractionated	0.992	0.116	41.8	0.111	0.121
	A	0.987	0.257	29.3	0.239	0.274
	B1	0.991	0.148	39.0	0.140	0.155
	B2	0.999	0.008	12.9	0.007	0.009
RS	Un-fractionated	0.997	0.048	51.7	0.046	0.050
	A	0.969	0.211	21.9	0.192	0.230
	B1	0.995	0.057	47.2	0.055	0.059
	B2	0.997	0.005	4.4	0.003	0.007
Corn	Un-fractionated	0.995	0.182	48.5	0.175	0.190
	A+B1	0.996	0.187	53.7	0.180	0.194
SH	Un-fractionated	0.963	0.060	20.4	0.054	0.066
	A	0.975	0.290	27.7	0.269	0.310
	B1	0.828	0.038	5.7	0.025	0.051
	B2	0.997	0.138	58.4	0.133	0.142
SBM	Un-fractionated	0.998	0.096	92.1	0.094	0.098
	A	0.987	0.243	36.8	0.230	0.256
	B1	0.992	0.050	32.6	0.047	0.053
CMLP	Unfractionated	0.938	0.134	23.5	0.122	0.145
	A	0.950	0.126	26.3	0.117	0.136
	B1	0.903	0.147	18.5	0.131	0.163

CS = Corn silage; RS = Rice straw; SH = Soybean hull; SBM = Soybean meal; CMLP = Cell mass from lysine production.

fraction was lower than their value; however, it was hard to compare our value directly with their value for the B2 fraction because they used a two-pool logistic model to estimate Kd. The Kd of carbohydrate fractions of rice straw was 4.8%/h for un-fractionated, 21.1%/h for A fraction, 5.7%/h for B1 fraction, and 0.5%/h for B2 fraction. Kd of the B2 fraction was poorly defined with a t-value of 4.4 and 95% confidence interval between 0.3 to 0.7%. The t-values of the other fractions were higher than 21.9. The slower degradation rate of rice straw, especially of fraction B1 and B2, than corn silage indicates its low quality. Corn showed the highest Kd in this study, as expected. Kd value was 18.2 and 18.7%/h for un-fractionated and A plus B1 fractions. Only a small amount of gas was produced from NDF. Corn grain contains less than 3% of sugar in dry matter (Chen et al., 1999) and pool size of the A fraction was also small (Table 1). Thus, an attempt to combine fraction A and B1 into the neutral detergent soluble fraction, as described by Chen et al. (1999), would be reasonable to analyze the digestion kinetics of carbohydrate in corn. The rate constant obtained by the approach was similar to that of un-fractionated corn grain because there was no effect from the B2 fraction. Large Kd of carbohydrate fractions were observed in soybean hull: 6.0, 29.0, 3.8, 13.8%/h for un-fractionated, A, B1 and B2 fractions, respectively. The t-values were higher than 20 except for the B1 fraction (5.7). The large Kd of fraction B2 indicates that the NDF in soybean hull can be easily degraded in the rumen, and this also underlines the importance of understanding digestion kinetics of each feedstuff. The same nutrient, analyzed by commonly used chemical techniques, in different feeds may not have the same degradation characteristics in the rumen due to different composition, linkage, and other effects. Kd values of soybean meal were 9.6%/h for the un-fractionated, 24.3%/h for the A fraction, and 5.0%/h for the B1 fraction. In the fermentation of CMLP, the Kd values were 13.4, 12.6 and 14.7%/h for un-fractionated, A and B1 fractions, respectively; however, the gas production was too low to be measured accurately.

In conclusion, the automated gas production system used in the present study was found to be useful in obtaining gas measurements for a non-linear mathematical model and in estimating well-defined kinetic parameters. Moreover, the system can be one of the best methods for estimation of Kd of soluble carbohydrates. However the subtraction method showed some limitations in its use for a wide range of feed ingredients besides forages, and the gas production technique may not a good method for application to feeds high in protein because nitrogen-containing compounds can interfere with gas production.

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