

# Estimation of Digestibility from *In Vitro* Rumen Fermentation Data in Some Forages of Puerto Rico

José A. Arroyo-Aguilú<sup>1</sup>

## INTRODUCTION

The present study was undertaken as an attempt to estimate *in vivo* forage energy digestibility from *in vitro* cellulose digestion (I.V.C.D.) in some forages of Puerto Rico. High correlations between measures of *in vivo* and *in vitro* digestion of forage crops have been found by Hershberger *et al.* (10)<sup>2</sup>, Donefer *et al.* (8), Baumgardt *et al.* (5), Naga and El-Shazly (12), Reid *et al.* (14), Arroyo-Aguilú *et al.* (1), and Chalupa and Lee, Jr. (6). As a result, I.V.C.D. is being widely used as a biological approach to forage nutritive evaluation. This technique is accurate, simple, less costly, and less time-consuming than *in vivo* evaluations.

## MATERIALS AND METHODS

Forage samples of known nutritive value (4), as determined in conventional digestion trials with dairy cattle (3), were used in this study to evaluate the *in vitro* procedure. The forages used were Merker grass (*Pennisetum purpureum*), Guinea grass hybrid (*Panicum maximum*), and Buffel grass (*Cenchrus ciliaris*) harvested at 49 to 55 and at 63 to 69 days of age at the Gurabo Substation farm. The grasses were fertilized at the rate of 400 pounds of N, 300 of P<sub>2</sub>O<sub>5</sub>, and 300 of K<sub>2</sub>O per acre per year. Forty-two samples, seven from each grass, and six composite samples of these same grasses were included in each run.

One-gram samples of ground dry forage were weighed into 100-ml. lipless beakers to serve as artificial rumina (1). Twenty milliliters of the filtered rumen-fluid inoculum and 30 ml. of a phosphate nutrient solution (1), were added to each beaker and mixed gently with the hay substrate.

Carbon dioxide was passed over the surface of the beaker contents. Immediately the beakers were closed with stoppers, equipped with Bunsen-type gas-release valves, and fermented in an incubator at 39°C.

Two beakers, one with a 0.5-gm. sample of crystalline cellulose<sup>3</sup> and the other with a 40-ml. rumen-fluid sample, were always included in each run.

<sup>1</sup> Assistant Nutritionist, Animal Husbandry Department, Agricultural Experiment Station, University of Puerto Rico, Río Piedras, P.R. The author wishes to express his gratitude to Dr. Luis Rivera-Brenes, Head of the Animal Husbandry Department of this Station, for his cooperation during the initiation of this study, and to the Plant Breeding Department of this Station for supplying the vegetative material used in this study.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 139.

<sup>3</sup> Avicel used is a product of American Viscose Corp., Marcus Hook, Pa. Trade name is used to provide factual information. This does not imply guarantee, warranty, or endorsement.

The artificial-rumen samples were incubated for 12-, 24-, 36-, and 48-hour periods on 3 days to account for day-to-day variation. Fermentation periods of 60 and 72 hours were also conducted on duplicate forage samples using rumen-fluid inoculum from two fistulated animals (2).

At the end of the fermentation periods, 1 ml. of 2 N H<sub>2</sub>SO<sub>4</sub> was added to each beaker to stop microbial activity. The samples were then dried in an oven at 65°C.

Cellulose was determined by the Matrone modification (11) of the Crampton and Maynard procedure (7). I.V.C.D. coefficients (table 1) were calculated according to Arroyo-Aguilú *et al.* (1).

Two rumen-fistulated cows fitted with permanent rumen-cannulas (2)

TABLE 1.—Average percentage *in vitro* cellulose digestion data for 3 forage grasses of known nutritive value

Forage grass	Age	Data on <i>in vitro</i> cellulose digestion, in hours—					
		12	24	36	48	60	72
	<i>Days</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Merker	49-55	17.25	31.79	42.90	47.86	46.77	45.44
Buffel	do.	11.63	27.33	36.82	42.71	46.97	46.07
Guinea	do.	15.57	32.31	42.20	45.47	49.84	48.65
Merker	63-9	13.22	25.76	35.41	43.14	45.27	46.56
Buffel	do.	6.32	24.07	30.77	32.66	40.00	39.32
Guinea	do.	13.27	26.83	34.00	40.05	40.37	43.78

served as the sources of rumen-fluid inoculum. Both animals were fed daily grass hay *ad libitum*.

Correlation and regression analyses were made according to the method of Steel and Torrie (15).

## RESULTS AND DISCUSSION

The rates of percentage I.V.C.D. for 12-, 24-, 36-, and 48-hour fermentation periods (table 1) were correlated with percentages of digestible dry matter (D.D.M.), total digestible nutrients (T.D.N.), digestible energy (D.E.), and nutritive value index (N.V.I.) as shown in table 2. Percentage relative intake (R.I.) and percentage voluntary intake (V.I.) were correlated with 12- and 24-hour I.V.C.D. rates, respectively. I.V.C.D. rates were pooled and are presented in figure 1.

These grasses were evaluated *in vitro* up to 72-hour fermentation periods (fig. 1). However, correlation analyses and regression equations (15) were computed between *in vivo* data and *in vitro* values obtained in fermentation



periods of 12, 24, 36, and 48 hours only. Mold growth was evident at 60 hours on the substrate surface.

Low correlations ( $r$ ) between 12-hour I.V.C.D. values and D.D.M. ( $r = 0.76$ ), T.D.N. ( $r = 0.64$ ), N.V.I. ( $r = 0.64$ ), and D.E. ( $r = 0.60$ ) were obtained. In New Jersey, Arroyo-Aguilú *et al.* (1) found also a nonsignificant correlation ( $r = 0.41$ ) between 12-hour I.V.C.D. and N.V.I. This might

TABLE 2.—Regression equations and correlation coefficients for estimating forage nutritive value from *in vitro* forage cellulose digestion

Variable correlated <sup>1</sup>	Regression equations	Correlation coefficients <sup>2</sup> ( $r$ )
D.D.M. (Y) with—		
12-hour I.V.C.D.	$Y = 42.41 + 1.239X$	0.76
24-hour I.V.C.D.	$Y = 12.39 + 1.641X$	.89*
36-hour I.V.C.D.	$Y = 12.84 + 1.230X$	.95**
48-hour I.V.C.D.	$Y = 16.01 + 1.009X$	.87*
T.D.N. (Y) with—		
12-hour I.V.C.D.	$Y = 40.14 + .869X$	.64
24-hour I.V.C.D.	$Y = 14.88 + 1.301X$	.84*
36-hour I.V.C.D.	$Y = 17.79 + .906X$	.94**
48-hour I.V.C.D.	$Y = 20.39 + .737X$	.76
D.E. (Y) with—		
12-hour I.V.C.D.	$Y = 74.90 + 1.552X$	.60
24-hour I.V.C.D.	$Y = 2.461X - 14.05$	.83*
36-hour I.V.C.D.	$Y = 1.801X - 11.76$	.94**
48-hour I.V.C.D.	$Y = 1.370X - 2.60$	.73
N.V.I. (Y) with—		
12-hour I.V.C.D.	$Y = 43.84 + .521X$	.64
24-hour I.V.C.D.	$Y = 22.53 + .998X$	.74
36-hour I.V.C.D.	$Y = 22.34 + .762X$	.81*
48-hour I.V.C.D.	$Y = 20.49 + .716X$	.85*

<sup>1</sup> See text pp. 133-4 for explanation of abbreviations.

<sup>2</sup> Significant at the 5-percent level of probability ( $P < 0.05$ ); \*\* Significant at the 1-percent level of probability ( $P < 0.01$ ).

have been because within-trial variability is greatest at this fermentation period. Donefer *et al.* (8) and Chalupa and Lee, Jr. (6) reported significant correlations between 12-hour I.V.C.D. and N.V.I.

No relationship between *ad libitum* intake (R.I. and V.I.) with 12- and 24-hour I.V.C.D. rates was obtained. The lag periods at early stages of incubation, the shock in transfer of the micro-organisms from the animal to the *in vitro* system, and the poor initial availability of the nutrients could possibly slow down the rate of I.V.C.D. At 12-hour periods, results were rather inconsistent and variable, and probably affected the 24-hour period somewhat. Reid *et al.* (14) observed no relationship between V.I.

and I.V.C.D. Nevertheless, Chalupa and Lee, Jr. (6) obtained high correlations between R.I. and 12- and 18-hour I.V.C.D. Further work and more data are probably needed to clarify this phase of the *in vitro* work.

Significant correlations between 24-hour I.V.C.D. and D.D.M. ( $r =$

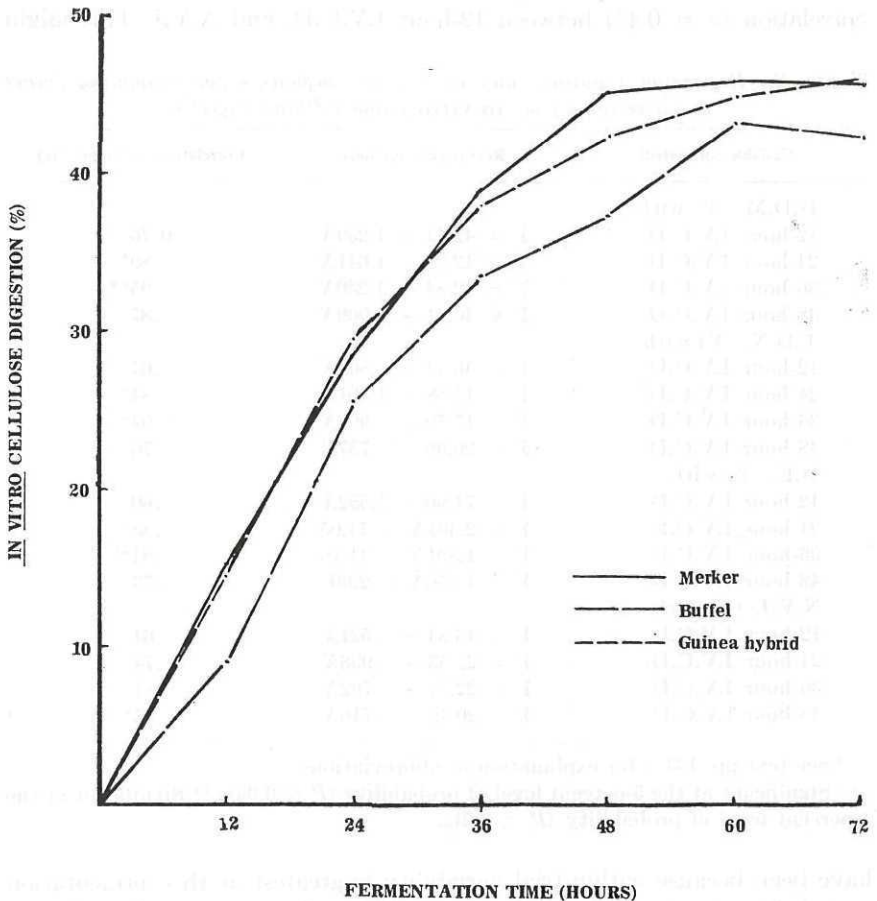


FIG. 1.—Rate of *in vitro* cellulose digestion of Merker, Guinea hybrid, and Buffel grasses.

0.89), T.D.N. ( $r = 0.84$ ) and D.E. ( $r = 0.83$ ) were obtained. I.V.C.D at 24 hours was nonsignificantly correlated with N.V.I. ( $r = 0.74$ ). D.E. correlations with 24-hour I.V.C.D. were reported as highly significant by Hershberger *et al.* (10), Naga and El-Shazly (12), Baumgardt *et al.* (5), Donefer *et al.* (8), and Arroyo-Aguilú *et al.* (1). Chalupa and Lee, Jr., (6) significantly correlated 24-hour I.V.C.D. with both D.E. and T.D.N.

Arroyo-Aguilú *et al.* (1) obtained, with 24-hour I.V.C.D., highly significant correlations for T.D.N. but significant correlations for N.V.I. Lag-time differences were still observed at incubation periods of 24 hours, although good correlations were obtained.

Thirty-six-hour I.V.C.D. values were highly significantly correlated with D.D.M. ( $r = 0.95$ ), D.E. ( $r = 0.94$ ) and T.D.N. ( $r = 0.94$ ). N.V.I. ( $r = 0.81$ ) was significantly correlated with I.V.C.D. at 36 hours. These correlations suggest that 36-hour incubation periods may offer the best estimates of energy availability. Fermentation periods of approximately 36 hours seem to be a measure of total digestion. Highly significant correlations between I.V.C.D. at 36 hours and D.D.M. or D.E. were obtained by Reid *et al.* (14).

Correlations between D.D.M. ( $r = 0.87$ ) and N.V.I. ( $r = 0.85$ ) with 48-hour I.V.C.D. were found to be significant. I.V.C.D. at 48 hours was found to be nonsignificant with both T.D.N. ( $r = 0.76$ ) and D.E. ( $r = 0.73$ ). Reid *et al.* (14) found I.V.C.D. between 24 and 48 hours to be highly correlated with D.D.M.

Maximum cellulose digestion was apparently obtained at 60 hours (fig. 1) for four of the six forages studied. The remaining two grasses, Merker and Guinea at 63 to 69 days of age, increased their I.V.C.D. values at 72 hours by 1.29 and 3.41 percentage units, respectively. This is in agreement with the results of Quicke *et al.* (13), in which maximum cellulose digestion of more mature forages was obtained with a longer fermentation period.

No marked differences in 60- and 72-hour I.V.C.D. values were observed when rumen-fluid inoculum from two different sources was used. Donefer *et al.* (9) obtained similar results in cellulolytic response when inoculum donor steers were fed the same forage.

#### SUMMARY AND CONCLUSIONS

Forty-two samples (seven from each grass) and six composite samples of Merker grass (*Pennisetum purpureum*), Guinea grass hybrid (*Panicum maximum*), and Buffel grass (*Cenchrus ciliaris*) were used in this study to evaluate the artificial rumen, or *in vitro* cellulose digestion, technique (1). These forage samples of known nutritive value (4), as determined in conventional digestion trials with cattle (3), were harvested at 49 to 55 and at 63 to 69 days of age at the Gurabo Substation farm.

No relationship was found between *ad libitum* intake (R.I. or V.I.) and 12- and 24-hour I.V.C.D. Within-trial variability due to lag-time differences could probably account for this.

I.V.C.D. at 36 hours may be used as the best estimate of forage digestibility in terms of D.D.M. ( $r = 0.95$ ), D.E. ( $r = 0.94$ ), and T.D.N. ( $r = 0.94$ ). Forty-eight-hour I.V.C.D. values could also be used as a predictor



of forage digestibility in terms of D.D.M. ( $r = 0.87$ ) and N.V.I. ( $r = 0.85$ ). Nevertheless, the highest correlations were obtained with *in vitro* values at 36 hours. There was a tendency for correlation coefficients to increase up to 36-hour digestion periods with increasing fermentation rates. From the data in the present study, the regression equations to predict *in vitro* D.D.M. ( $Y$ ), *in vitro* D.E. ( $Y$ ), and *in vitro* T.D.N. ( $Y$ ) from 36-hour I.V.C.D. ( $X$ ) were  $Y = 12.84 + 1.23 X$ ,  $Y = 1.801 X - 11.76$ , and  $Y = 17.79 + 0.906 X$ , respectively.

Maximum cellulose digestion was apparently obtained at 60 hours for four of the six forages studied. When two inoculum donor animals were fed the same forage, no marked differences in 60- and 72-hour I.V.C.D. values were observed.

### RESUMEN Y CONCLUSIONES

Cuarenta y dos muestras (siete de cada yerba) y seis muestras compuestas de yerba Merker (*Pennisetum purpureum*), híbrido de la yerba Guinea (*Panicum maximum*) y de yerba Buffel (*Cenchrus ciliaris*) se usaron en este estudio para evaluar la técnica del rumen artificial, o sea, la técnica de la digestión de celulosa *in vitro* (1). Estas muestras de forrajeras cuyo valor nutritivo es conocido (4), y además, ya determinado en pruebas convencionales de digestibilidad con ganado vacuno (3), fueron cosechadas de los 49 a los 55 días y de los 63 a los 69 días de haberse sembrado en la Subestación de Gurabo.

No se estableció relación alguna entre el consumo *ad libitum* (consumo relativo—C.R. o consumo voluntario—C.V.) y la digestión de la celulosa *in vitro* (D.C.I.V.) a las 12 y a las 24 horas. La variabilidad dentro de las pruebas, debido a las diferencias en los períodos de retraso, podría ser responsable de esto.

La D.C.I.V. a las 36 horas puede usarse para estimar la digestibilidad forrajera en términos de materia seca digestible (M.S.D.) ( $r = 0.95$ ), energía digestible (E.D.) ( $r = 0.94$ ), y nutrimentos digestibles totales (N.D.T.) ( $r = 0.94$ ). La D.C.I.V. a las 48 horas también puede usarse para predecir la digestibilidad en términos de materia seca digestible (M.S.D.) ( $r = 0.87$ ) e índice de valor nutritivo (I.V.N.) ( $r = 0.85$ ). Sin embargo, las correlaciones más altas se obtuvieron con valores *in vitro* a las 36 horas. Existió una tendencia de los coeficientes de correlación a aumentar hasta las 36 horas de fermentación, según aumentaba la D.C.I.V.

Se calcularon las siguientes ecuaciones de regresión para predecir M.S.D. *in vitro* ( $Y$ ), E.D. *in vitro* ( $Y$ ) y N.D.T. *in vitro* ( $Y$ ) de la D.C.I.V. a las 36 horas ( $X$ ):  $Y = 12.84 + 1.23 X$ ,  $Y = 1.801 X - 11.76$  é  $Y = 17.79 + 0.906 X$ , respectivamente.

La digestión máxima de la celulosa se obtuvo aparentemente a las 60

horas en cuatro de las seis yerbas forrajeras estudiadas. Cuando se alimentaron dos animales con la misma clase de forraje, no se encontraron diferencias marcadas en los valores de la D.C.I.V. a las 60 y a las 72 horas.

#### LITERATURE CITED

1. Arroyo-Aguilú, J. A., Evans, J. L., and Taylor, M. W., The artificial-rumen technique for estimating the nutritive value of forages, *J. Agr. Univ. P.R.* 47(3): 169-79, 1963.
2. Arroyo-Aguilú, J. A., and Rivera-Anaya, J. D., Plastic cannula for rumen fistula, *J. Agr. Univ. P.R.* 49(2): 269-70, 1965.
3. Arroyo-Aguilú, J. A., and Rivera-Brenes, L., Digestibility studies on Napier (Merker) grass (*Pennisetum purpureum*), Giant Pangola grass (*Digitaria valida* Stent.), and Signal grass (*Brachiaria brizantha*), *J. Agr. Univ. P.R.* 45(33): 151-6, 1961.
4. Arroyo-Aguilú, J. A., Rivera-Brenes, L., Acosta-Matienzo, A., and Carrero, R., The nutritive evaluation of Buffel grass (*Cenchrus ciliaris*) and Guinea grass hybrid (*Panicum maximum*) as compared to Napier (Merker) grass (*Pennisetum purpureum*), *J. Agr. Univ. P.R.* (in press).
5. Baumgardt, B. R., Taylor, M. W., and Cason, J. L., Evaluation of forages in the laboratory, II, Simplified artificial rumen procedure for obtaining repeatable estimates of forage nutritive value, *J. Dairy Sci.* 45: 62-7, 1962.
6. Chalupa, W., and Lee, Jr., D. D., Estimation of forage nutritive value from *in vitro* cellulose digestion, *J. Dairy Sci.* 49: 188-92, 1966.
7. Crampton, E. W., and Maynard, L. A., The relation of cellulose and lignin content to the nutritive value of animal feeds, *J. Nutr.* 15: 383-95, 1938.
8. Donefer, E., Crampton, E. W., and Lloyd, L. E., Prediction of the nutritive value index of a forage from *in vitro* rumen fermentation data, *J. Anim. Sci.* 19: 545-52, 1960.
9. Donefer, E., Lloyd, L. E., and Crampton, E. W. Effect of several variables of the *in vitro* digestion of forage cellulose, *J. Anim. Sci.* 20: 959, 1961.
10. Hershberger, T. V., Long, T. A., Hartsook, E. W., and Swift, R. W., Use of the artificial rumen technique to estimate the nutritive value of forages, *J. Anim. Sci.* 18: 770-9, 1959.
11. Matrone, G., A. Study of Lignin and Cellulose Methods for the Chemical Evaluation of Feeds, M.S. Thesis, Cornell University, Ithaca, N.Y., 1944.
12. Naga, M. M. A., and El-Shazly, K., The use of *in vitro* fermentation technique to estimate the digestible energy content of some Egyptian forages, I. The *in vitro* digestion of cellulose as a criterion of energy content, *J. Agr. Sci.* 61: 73-9, 1963.
13. Quicke, G. V., Bentley, O. G., Scott, H. W., and Moxon, A. L., Cellulose digestion *in vitro* as a measure of the digestibility of forage cellulose in ruminants, *J. Anim. Sci.* 18: 275-87, 1959.
14. Reid, R. L., Clark, B., Welch, J. A., Jung, G. A., and Shelton, D. C., Relationship of forage digestibility and intake data to *in vitro* and *in vivo* fermentation indices, *J. Anim. Sci.* 19: 1312, 1960.
15. Steel, R. G. D., and Torrie, J. H., Principles and Procedures of Statistics, McGraw-Hill Book Co., Inc., New York, N.Y., 1960.