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Evaluation of Two In Vitro Fermentation Methods for Estimating the Nutritive Value of Mixed Rations for Beef Cattle

University of Tennessee Agricultural Experiment Station

K. M. Barth

A. S. Mohammed

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The University of Tennessee
Agricultural Experiment Station
John A. Ewing, Director
Knoxville

SUMMARY

SAMPLES of 11 mixed rations on which steer digestion and intake data were available were selected for *in vitro* fermentation experiments. Two inoculation methods were used (whole rumen liquor method and washed-cell suspension method). Both *in vitro* dry matter digestibility and cellulose digestibility were determined. Comparison of the results with those from the *in vivo* steer experiments led to the following conclusions:

1. *In vitro* 24-hour fermentation cellulose digestiblities from both artificial rumen methods were not highly correlated with the four expressions of *in vivo* digestibility (DDM, DOM, TDN and DE), and should not be used to predict the digestibility of mixed rations for beef cattle.
2. When *in vitro* dry-matter digestibility was considered, the results of the washed-cell suspension method were more highly correlated with digestibility than results from the whole-rumen liquor method.
3. In predicting the digestibility of mixed rations, the method of choice would seem to be the 24-hour *in vitro* dry-matter digestibility from the washed-cell suspension method, the highly significant correlations of which were 0.76 with TDN and 0.83 with DE.
4. The short-term fermentation periods (8 and 12 hours) from the whole-rumen liquor method were not highly correlated with VI.
5. With the washed-cell suspension method, both 8-hour *in vitro* dry-matter digestibility and 12-hour *in vitro* cellulose digestibility were significantly correlated with VI. The 8-hour *in vitro* dry-matter digestibility from the washed-cell suspension method was more highly correlated with VI and is more easily determined than cellulose digestibility.

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K. M. Barth and A. S. Mohammed [1/]

INTRODUCTION

SEVERAL laboratory (*in vitro*) methods have been proposed for estimating the nutritive value of forages. They are less costly, less time-consuming, and require a smaller amount of test material than experiments with animals. However, these laboratory methods will not completely replace animal (*in vivo*) experimentation. At best, they are an estimate of the results that would be obtained from animals, and the accuracy with which these methods indicate the value of feeds for animals has to be determined before they can be used to advantage.

Many laboratory techniques and modifications of techniques have been developed in which *in vitro* dry-matter or cellulose digestion was determined to estimate either *in vivo* total digestible nutrients, digestible energy, or digestible dry matter. Early *in vitro* techniques involved the use of whole-rumen liquid as inoculum (Marston, 1948; Louw *et al.*, 1949; Burroughs *et al.*, 1950). These workers indicated that this method of forage evaluation might best be used as a screening device of important factors influencing rumen physiology from which the most promising results and actual applications can be checked in animal experimentations.

An alternate *in vitro* technique was developed by Cheng *et al.* (1955). Instead of using whole-rumen liquid as inoculum, washed suspensions of rumen microorgranisms were used. They stated that this alternate *in vitro* method "is more applicable in determining requirements of rumen microorganisms such as minerals, vitamins, and unidentified growth factors, in the elucidation of biochemical reactions in the rumen, and in the study of

1/Assistant Professor of Animal Husbandry and former Research Assistant in the Animal Husbandry - Veterinary Science Department, respectively.

conditions necessary for maximum utilization of cellulose by rumen microorganisms." However, Van Dyne (1962) reported that, for the prediction of nutritive value of forages, the whole-rumen liquid method was comparable to more elaborate procedures of processing the inoculum, such as the washed - cell method.

In addition to the estimation of forage digestibility by animals, there recently has been an increased interest in measuring the amount of forage that an animal voluntarily consumes. In fact, it has been shown by several workers (Crampton, 1957; Byers and Ormiston, 1962; and Ingalls *et al.,* 1965) that the intake of a forage has a greater effect on animal performance than its digestibility. Therefore, Crampton *et al.* (1960) proposed that a good predictor of animal performance was the product of forage intake and the energy digestibility of the forage, which they termed 'Nutritive Value Index.' The same research group (Donefer *et al.,* 1960) observed that this Nutritive Value Index was highly correlated with 12-hour *in vitro* cellulose digestion. High coefficients of correlation between forage intake and short-period *in vitro* digestibility were reported by Bratzler (1961), Johnson *et al.* (1962), Karn *et al.* (1964), and Chalupa and Lee (1966).

So far, most *in vitro* fermentation studies have been performed on forages alone. However, in many instances forages make up only part of the ration of ruminant animals. Therefore, the objective of the present study was to determine how well short-period *in vitro* fermentation data predict mixed ration intake and how well long-period fermentation data predict mixed ration digestibility, using two *in vitro* fermentation methods, the whole-rumen liquor method and the washed-cell suspension method.

EXPERIMENTAL PROCEDURE

THREE replicate *in vitro* fermentaton experiments were conducted. Each experiment consisted of 66 treatments involving 11 ration samples, 2 inoculation methods, and 3 fermentation periods. Duplicate flasks were run for each treatment.

Forages. Samples of rations for which the nutritive values had been determined in digestion trials and feedlot trials with beef cattle were used in this study. The feedlot and digestibility data were those reported by B.B. Wilson, Jr. (1964); G. R. Wil-

son (1964); Mohammed *et al.* (1967); and Barth and Prigge (1967). A summary of the results obtained from these trials is given in Appendix Tables 1 and 2.

Samples from the ration ingredients were collected during the feedlot trials and were ground to pass through a 40-mesh screen. Four-tenths gram of the ground forage plus the appropriate amount of ground cottonseed meal and/or concentrate mixture were placed in 125-milliliter Erlenmeyer fermentation flasks. The ratio of cottonseed meal and/or concentrate to forage on a dry-matter basis was the same as in the feedlot trials.

Whole-Rumen Liquor Method. Rumen contents were obtained from a rumen-fistulated beef steer that had been fed good quality alfalfa hay. The material was strained through eight layers of cheesecloth into a previously-warmed thermos bottle. In the laboratory, the liquid was re-strained through cheesecloth and 15 milliliters were pipetted into each fermentation flask which contained 0.4 gram of the feed material and 15 milliliters of a carbonate buffer.

Washed-Cell Suspension Method. A washed-cell suspension was prepared from the strained rumen liquor according to the method of Cheng *et al.* (1955). The liquor was passed through a high-speed centrifuge (Sharples super-centrifuge) to separate the bacteria from the liquid and feed debris. Rumen bacteria were washed twice in a sodium bicarbonate solution and 15-milliliter portions of the bacterial buffer suspension were added to each 125-milliliter Erlenmeyer flask, which contained 0.4 gram of the feed material and 15 milliliters of a carbonate buffer.

Both Fermentation Methods. The flasks were placed in a water bath kept at 39 degrees C. Carbon dioxide gas was bubbled through the contents of the flasks throughout the fermentation period to maintain an anaerobic condition. Fermentation lasted for either 8, 12, or 24 hours. After this, the microbial action was stopped by adding 1 milliliter of glacial acetic acid. The samples were dried and the *in vitro* dry-matter digestion was determined. In addition, the cellulose content of the rations was determined before and after fermentation, using the method of Crampton and Maynard (1938), and cellulose digestion was calculated.

Correlation Between Animal and Laboratory Data. All laboratory methods of feed evaluation which were used in this

study were compared with *in vivo* digestibility and voluntary intake to determine how well they estimate the nutritive value of the feed.

Ration digestibility obtained from the digestion trials was expressed as either dry-matter digestibility (DDM), organic matter digestibility (DOM), total digestible nutrients (TDN), or digestible energy (DE).

Voluntary Intake was expressed either on the basis of the feed consumption per 100 pounds of body weight or on the basis of metabolic body size ($W_{Kg.}^{0.75}$). The four expressions of in *vivo* digestibility (DDM, DOM, TDN and DE) and the voluntary intake data (calculated in two ways) were then correlated with the laboratory results from the two fermentation methods (whole-rumen liquor and washed-cell suspension), two expressions of *in vitro* digestibility (dry-matter and cellulose), and three fermentation times (8, 12, and 24 hours.)

RESULTS AND DISCUSSION

THE dry-matter and cellulose digestion coefficients obtained from the two *in vitro* fermentation methods are reported in Table 1 while results from the analyses of variance are presented in Tables 2 and 3. Both methods ranked the various rations in approximately the same order regarding dry-matter and cellulose digestion.

However, the percent of dry matter and cellulose digested by the whole-rumen liquor method was significantly higher ($P < .01$) than with the washed-cell suspension method. As expected, significant differences ($P < .01$) were observed between dry-matter and cellulose digestiblity after the 8-, 12-, and 24-hour periods of fermentation. There was also an interaction ($P < .01$) of digestion time with method in dry-matter digestion, and of digestion time with both method and ration in cellulose digestion.

Digestibility Correlations. The coefficients of correlation between each of the four *in vivo* digestibility measures (DDM, DOM, TDN, and DE) and the 24-hour dry-matter and cellulose digestibilities *in vitro* are presented in Table 4.

In the whole-rumen liquor method, the coefficients of correlation between the four *in vivo* digestibility measures and 24-hour *in vitro* dry-matter digestibility generally were low and

Table 1. In vitro dry matter and cellulose digestion [a]

Ration number	Fermentation time (Hrs.)	Whole rumen liquor method		Washed cell suspension method	
		% Dry matter digested	% Cellulose digested	% Dry matter digested	% Cellulose digested
1	8	47.6	29.2	42.9	25.3
	12	55.7	39.2	48.4	38.3
	24	67.7	51.2	54.5	49.7
2	8	43.9	26.3	40.3	23.0
	12	53.6	34.1	45.9	33.0
	24	64.1	48.5	52.3	48.3
3	8	34.9	18.9	28.8	17.6
	12	42.1	26.5	35.4	21.2
	24	54.7	37.8	39.5	38.6
4	8	35.4	25.9	31.5	23.0
	12	43.6	36.5	36.4	32.5
	24	58.0	41.0	42.0	41.3
5	8	33.8	24.7	30.3	21.7
	12	41.9	32.2	34.7	30.1
	24	56.4	39.3	40.9	40.1
6	8	37.7	29.1	33.3	25.1
	12	46.9	36.1	40.8	34.9
	24	67.3	40.3	45.4	39.6
7	8	35.3	26.8	31.4	22.6
	12	43.3	34.2	37.7	30.6
	24	57.7	37.3	41.7	37.0
8	8	40.9	26.7	36.9	22.9
	12	51.3	37.3	41.6	37.7
	24	65.8	48.2	49.0	47.9
9	8	40.6	24.9	34.6	21.5
	12	49.9	35.8	38.6	36.3
	24	63.5	46.7	46.7	46.8
10	8	39.8	24.3	33.5	21.2
	12	49.3	35.0	37.5	34.8
	24	63.1	45.9	45.1	45.3
11	8	35.7	22.1	31.2	19.5
	12	42.8	31.7	35.4	33.0
	24	54.7	41.1	41.2	42.2

[a] Each value is the mean of six individual determinations.

Table 2. Analysis of variance of in vitro dry matter digestion

Source	d.f.	S.S.	M.S.	F.
Total	197			
Rations (R)	10	3563.8	356.3	73.0**
Methods (M)	1	4474.9	4474.9	917.6**
Digestion times (D)	2	9517.9	4758.9	975.8**
R x M	10	75.4	7.5	1.5
R x D	20	62.2	3.1	0.6
M x D	2	1107.2	553.6	113.5**
Error	152		4.8	

Table 3. Analysis of variance of in vitro cellulose digestion

Source	d.f.	S.S.	M.S.	F.
Total	197			
Rations (R)	10	1889.9	188.9	115.1**
Methods (M)	1	123.6	123.6	75.3**
Digestion times (D)	2	12664.5	6332.2	3859.0**
R x M	10	23.8	2.3	1.4
R x D	20	712.6	35.6	21.7**
M x D	2	86.5	43.2	26.3**
Error	152		1.6	

non-significant (Table 4). These coefficients obtained from mixed rations are in contrast to the high and statistically significant coefficients obtained from forages by Smith *et al.* (1965).

Correlations between 24-hour cellulose digestibility and *in vivo* digestibility measures were generally somewhat higher. The correlation with *in vivo* dry-matter digestibility (0.69) was statistically significant ($P < .05$). Therefore, with the whole-rumen liquor method, only 24-hour *in vitro* cellulose digestibility was a fair predictor of one of the *in vivo* digestibility measures of mixed rations.

Using one of the whole-rumen liquor methods, close relationships between various measures of *in vivo* forage digestibility on one hand, and long-period *in vitro* cellulose digestion on the other hand were reported by Kamstra *et al.* (1955), Pigden and Bell (1955), Barnett (1957), Hershberger *et al.* (1959), Reid *et al.* (1960), Donefer *et al.* (1960), Reid and Jung (1965), and Chalupa and Lee (1966).

When the washed-cell suspension method was used, however, the coefficients of correlation between the four expressions of *in vivo* digestibility (DDM, DOM, TDN, and DE) and 24-hour dry-matter and cellulose digestibility *in vitro* were somewhat higher (Table 4). The correlation between *in vivo* DDM and *in vitro* 24-hour cellulose digestion was 0.66 ($P < .05$) while the correlation coefficients of *in vitro* DDM with TDN and DE were 0.76 and 0.83, respectively. The last two highly significant coefficients indicate that 24-hour dry-matter digestion from the washed-cell suspension method is useful as a predictor of *in vivo* total digestible nutrients and digestible energy of mixed rations.

Table 4. Simple correlation coefficients between animal and laboratory methods which estimate the nutritive value of rations

In vitro digestibility %	Fermentation time (Hr.)	Dig. dry matter (%)	Dig. organic matter (%)	Total dig. nutr. (%)	Dig. Energy (kcal./gm.)	Vol. Intake [a]	Vol. Intake [b]
				Whole rumen liquor method			
Dry Matter	8	0.47	0.48	0.67*	0.82**	0.57	0.51
	12	0.08	0.32	0.50	0.60*	0.17	0.05
	24	0.05	0.29	0.45	0.56	0.15	0.01
Cellulose	8	0.06	0.19	0.38	0.50	0.06	-0.10
	12	0.02	0.27	0.55	0.61*	0.09	-0.01
	24	0.69*	0.47	0.45	0.47	0.67*	0.78**
				Washed cell supension method			
Dry Matter	8	0.62*	0.39	0.38	0.41	0.64*	0.73**
	12	0.44	0.48	0.69*	0.77**	0.50	0.49
	24	0.51	0.55	0.76**	0.83**	0.54	0.57
Cellulose	8	0.06	-0.18	0.26	-0.47	-0.09	0.14
	12	0.60*	0.54	0.45	0.73**	0.69*	0.51
	24	0.66*	0.59	0.41	0.59	0.65*	0.58

[a] Based on metabolic body size (gm. dry matter intake per kg body weight$^{.75}$).

[b] Based on body weight (lb. dry matter intake per 100 lb. body weight).

*Significant at the 0.05 level of probability.

**Significant at the 0.01 level of probability.

Although the 8- and 12-hour fermentation periods were chosen as predictors of Voluntary Intake or Nutritive Value Index, their coefficients of correlation with DE were, in some cases, as high or higher than the coefficients between DE and the 24-hour dry-matter and cellulose digestibility *in vitro* (Table 4).

With some exceptions, low and mostly non-significant correlation coefficients between the four measures of ration digestibility (DDM, DOM, TDN, and DE) and the 24-hour dry-matter or cellulose *in vitro* digestibility from both methods were obtained. The main reason for these low coefficients probably was the fact that the 11 forages in this study represented different plant species, hays, and silages, and usually were fed in combination with other ration constituents.

There were four acceptable predictors of *in vivo* digestibility among the various *in vitro* determinations. In both *in vitro* methods (washed-cell suspension and whole-rumen liquor), 24-hour *in vitro* cellulose digestion was significantly correlated with *in vivo* dry-matter digestibility. Also, the correlations of dry-matter digestion *in vitro* from the washed-cell suspension method with TDN and DE were highly significant.

The data indicate that some *in vitro* determinations yield usable predictors of digestibility, even when mixed rations and not forages alone were investigated. The best digestibility predictor was 24-hour *in vitro* dry-matter digestibility employing the washed-cell suspension method.

Voluntary Intake Correlations. The coefficients of correlation of the values from the 8-hour and 12-hour fermentation periods with Voluntary Intake are presented in Table 4. There was practically no difference between the results obtained from the two ways of calculating Voluntary Intake. In addition, the following general observations can be made:

1. Within the 8-hour fermentation time, *in vitro* dry-matter digestion was a more accurate predictor of Voluntary Intake than *in vitro* cellulose digestion.
2. Within the 12-hour fermentation time, results of the washed-cell suspension method predicted Voluntary Intake more accurately than results from the whole-rumen liquor method.

3. In 8-hour fermentation runs, the best predictor of Voluntary Intake was *in vitro* dry-matter digestibility from the washed-cell suspension method; and in the 12-hour fermentation runs, the best predictor was *in vitro* cellulose digestion also from the washed-cell method.

The coefficients of correlation between the 8-hour *in vitro* dry-matter digestion obtained from the washed-cell suspension method and Voluntary Intake based on metabolic size was 0.64 ($P < .05$). However, when Voluntary Intake was based on body weight, the correlation coefficient was 0.73 ($P < .01$). Also, Voluntary Intake based on metabolic size resulted in a significant correlation (0.69; $P < .05$) with 12-hour cellulose digestion obtained from the washed-cell suspension method.

Although the 24-hour values were considered to yield usable predictors of digestibility, and not for Voluntary Intake, significant correlation coefficients were obtained between cellulose digestibility and Voluntary Intake.

Short-term fermentation periods (up to 18 hours) have been shown to be good predictors of Voluntary Intake by Reid *et al.* (1960), Van Soest (1965), and Chalupa and Lee (1966). It should be reemphasized that in these studies forages alone were investigated, while in the present study mixed rations (forages and concentrates) were considered. Therefore, it appears that short-term *in vitro* dry-matter and cellulose digestibilities show promise in the prediction of Voluntary Intake even of mixed rations, although their predictive value is much lower than for forages.

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Appendix Table 1. Ration ingredients and their nutrient composition

Ration No.	Ingredient	Percent in ration [a]	Dry matter	Organic matter [b]	Crude Protein [b]	Ether extract [b]	Crude fiber [b]	N-free extract [b]	Ash [b]	Gross energy [b]
				%	%	%	%	%	%	kcal/gm.
1	good alfalfa hay	100.0	90.1	82.6	18.7	1.9	29.4	41.7	7.5	4.55
2	fair alfalfa hay	100.0	89.3	82.9	15.9	1.5	35.4	40.7	6.4	4.61
3	poor alfalfa hay	100.0	91.5	87.1	13.7	1.1	46.7	33.7	4.4	4.59
4	corn silage	48.0	30.8	26.4	8.0	2.6	25.5	59.5	4.4	4.58
	alfalfa hay	14.8	89.3	82.9	15.9	1.5	35.4	40.1	6.4	4.61
	concentrate mix [c]	37.2	89.2	86.7	17.9	4.0	4.5	71.1	2.5	4.65
5	nitrated corn silage	45.7	27.6	22.7	8.0	1.5	24.8	59.7	4.9	4.67
	alfalfa hay	15.5	89.3	82.9	15.9	2.8	35.4	40.1	6.4	3.61
	concentrate mix [c]	38.8	89.2	86.7	17.9	4.0	4.5	71.1	2.5	4.65
6	corn silage	75.4	26.3	22.7	9.0	2.7	24.0	60.7	3.6	4.46
	alfalfa-grass hay	16.4	89.7	81.6	15.0	2.9	35.4	38.7	8.1	4.46
	cottonseed meal	8.2	89.7	84.1	43.3	3.3	16.5	43.9	5.6	4.80
7	alfalfa silage	75.4	22.8	12.3	16.3	4.1	30.6	38.5	10.5	4.71
	alfalfa-grass hay	16.4	89.7	81.6	15.0	2.9	35.4	38.7	8.1	4.46
	cottonseed meal	8.2	89.7	84.1	34.3	3.3	16.5	43.9	5.6	4.80
8	1st cut corn silage [d]	67.3	23.3	17.3	11.1	1.4	24.7	56.9	6.0	4.40
	alfalfa hay	14.0	91.1	86.0	17.9	1.5	29.4	43.1	8.1	4.46
	cottonseed meal	18.7	94.9	93.7	40.3	3.6	14.1	40.7	1.2	4.94
9	2nd cut corn silage [d]	68.5	25.9	19.8	11.1	1.6	23.7	57.7	6.1	4.43
	alfalfa hay	13.5	94.1	86.0	17.9	1.5	29.4	43.1	8.1	4.46
	cottonseed meal	18.0	94.9	93.7	40.3	3.6	14.1	40.7	1.2	4.94

Appendix Table 1 (continued)

Ration No.	Ingredient	Percent in ration [a]	Dry matter	Organic matter [b]	Crude Protein [b]	Ether extract [b]	Crude fiber [b]	N-free extract [b]	Ash [b]	Gross energy [b]
				%	%	%	%	%	%	kcal/gm.
	3rd cut corn silage	70.1	29.8	23.2	10.5	1.8	24.1	57.1	6.6	4.38
10	alfalfa hay	12.8	94.1	86.0	17.9	1.5	29.4	43.1	8.1	4.46
	cottonseed meal	17.1	94.9	93.7	40.3	3.6	14.1	40.7	1.2	4.94
	4th cut corn silage	65.4	32.7	25.0	9.7	1.2	27.7	53.7	7.7	4.27
11	alfalfa hay	14.9	94.1	86.0	17.9	1.5	29.4	43.1	8.1	4.46
	cottonseed meal	19.8	94.9	93.7	40.3	3.6	14.1	40.7	1.2	4.94

[a] Feedlot and laboratory evaluations, dry matter basis.

[b] Dry matter basis.

[c] Four parts cracked corn and one part cottonseed meal.

[d] Refers to maturity of corn silage.

Appendix Table 2. In vivo digestibility and voluntary intake

Ration number	Voluntary Intake, body weight basis	Voluntary intake metabolic size basis	Dig. dry matter	Dig. org. matter	Total dig. nutri.	Dig. energy
			%	%	% nutr.	kcal/gm.
1	2.43	101.6	59.5	60.1	56.6	2.63
2	2.34	96.3	59.5	59.6	56.7	2.67
3	1.88	75.2	45.0	46.8	44.9	1.97
4	2.25	90.4	68.2	68.8	70.7	3.11
5	2.12	85.4	69.4	71.3	71.8	3.20
6	1.82	74.7	71.7	71.8	70.6	3.15
7	1.82	74.3	59.0	60.0	57.1	2.84
8	1.91	75.6	68.9	70.8	68.9	3.11
9	1.87	79.6	68.0	69.2	68.0	3.01
10	2.09	83.1	66.4	67.5	66.7	2.91
11	1.91	74.9	66.7	68.1	66.5	2.90

[a]/grams dry matter intake / 100 kg. body weight

[b]/grams dry matter intake / kg. body weight$^{.75}$

THE UNIVERSITY OF TENNESSEE
AGRICULTURAL EXPERIMENT STATION
KNOXVILLE, TENNESSEE

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