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A COMPARISON OF IN VITRO TECHNIQUES AND THEIR RELATION TO IN VIVO VALUES

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High correlations between the digestion of forages in the artificial rumen and in conventional digestion trials have been reported (Hershberger et al., 1959; LeFevre and Kamstra, 1960; Bowden and Church, 1962 b). In vitro procedures vary among laboratories, but basic components of all procedures are much the same. In a recent comparison of three in vitro methods, Barnes et al. (1964) found differences in digestion between methods after relatively short fermentation periods (6 - 12 hours) but not after longer periods (18 - 48 hours).

Simplification of in vitro methods would, in many cases, increase the utility of the artificial rumen. The work of Walker (1959) and Van Dyne (1962) would indicate that complexity of method is not a prerequisite for obtaining reliable in vitro digestion results.

The purpose of this research was to compare in vitro cellulose and dry matter digestion using 2 inoculum preparations and 2 fermentation periods. Main criteria for comparisons were variability and relation to in vivo digestion data.

EXPERIMENTAL PROCEDURE

Description of Forages. Twenty samples of native meadow hay representing 4 harvest dates ranging from early June to early August and 9 samples of dryland rye hay harvested at 3 stages of growth (flower, dough, and seed) were studied in the artificial rumen. Prior to this study, in vivo digestion values for these samples were established through trials with Columbia wethers.

Variables Studied. Two methods of inoculum preparation were compared: (1) strained rumen juice (S.R.J.) processed according to Bowden and Church (1962a) and mixed with CO_2-saturated basal medium buffer solution in a ratio of 1 part S.R.J. to 4 parts basal medium; and (2) resuspended microorganisms (R.M.). R.M. were prepared by centrifuging strained rumen liquor at 1,000 rpm for 3 minutes to remove plant debris, and then centrifuging the supernatant at 3,000 rpm for 30 minutes; the liquid was discarded and the microorganism cells were resuspended in 39° CO_2 saturated basal medium. Micro-

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organism cells were homogenized in a blender with a small amount (200 - 250 ml) of basal medium before adding the total amount of basal medium measured out for a particular run.

In vitro digestibility of dry matter and cellulose was determined for all samples after 24 and 48 hour incubation periods with each method of inoculum preparation. Separate trials (runs) were conducted for dry matter and cellulose at each incubation period with each method of inoculum preparation--making a total of 8 trials. During all trials each forage sample was included in duplicate fermentation vessels.

Inoculum Source and Collection. A 2 year-old fistulated Hereford steer maintained solely on native meadow hay was the source of rumen microorganisms. The steer was fitted with a 15 cm. plastic cannula which allowed access to the rumen by removal of a screw cap. Ingesta near the opening were removed and discarded in sufficient quantities to allow partial hand-mixing of rumen contents before sampling. Rumen liquor was squeezed from rumen contents through 2 layers of cheesecloth into a pre-warmed thermos. A second filteration through 4 layers of cheesecloth in the laboratory provided the S.R.J.

Fermentation Procedure. Fermentation was conducted in 50 ml. polyethylene centrifuge tubes maintained at 39°C in a water bath. Fermentation vessels were continually gassed with CO_2 at the rate of 40 - 50 bubbles per minute. Each vessel was fitted with a No. 5 1/2 rubber stopper equipped with inlet and outlet glass tubes to permit entrance and escape of CO_2 gas.

Forage samples were ground through a 40-mesh screen and weighed into fermentation tubes (250 mg. per tube). Twenty-five ml. of basal medium mixture, containing either S.R.J. or R.M., was added to each tube. The basal medium buffer solution used contained the following ingredients (g/liter distilled water): sodium phosphate, dibasic, 4.8; sodium bicarbonate, 4.8; potassium chloride, 0.7; and urea, 0.24. The pH of this mixture after CO_2 saturation and inoculum addition varied from 6.86 to 6.90; therefore no pH adjustments were made. Measurements of in vitro cellulose digestion followed those described by Quicke et al. (1959) while dry matter digestion was determined according to Bowden and Church (1962 a). Blank tubes were analyzed for each trial to correct for constituents added via the inoculum.

RESULTS AND DISCUSSION

Estimation of digestibility. Digestibility with sheep was underestimated by in vitro methods using 24 hours fermentation and overestimated using 48 hours fermentation (table 1). Similar findings were reported by LeFevre and Kamstra (1960) from a study comparing 22 rations. Quicke et al. (1959) however, found that 48 or 60-hour fermentations resulted in close agreement between in vitro and in vivo cellulose digestion.

Sheep trials conducted to measure the digestibility of meadow and rye forage were conducted in different trials, in different years, and with different age sheep; therefore, in vivo comparisons between the two forages probably do not reflect true differences (table 1)

Table 1. A comparison of *in vitro* techniques for estimating cellulose and dry matter digestibility obtained with animal digestion trials. 1/

Forage and harvest date or stage	Measurement	*In vitro* fermentation period and inocula preparation 2/				Digestibility in sheep trial, %
		24 hr.		48 hr.		
		R.M.	S.R.J.	R.M.	S.R.J.	
		%	%	%	%	%
Meadow hay:						
6/9	Cell.	59.1	64.6	77.2	79.2	68.0
	D.M.	53.9	61.4	69.0	72.2	61.8
6/28	Cell.	46.2	53.7	65.4	70.9	59.8
	D.M.	46.0	53.0	59.7	63.7	56.6
7/17	Cell.	42.9	49.0	61.7	67.0	55.2
	D.M.	42.2	47.2	54.0	58.8	51.7
8/4	Cell.	39.7	48.6	60.7	66.9	54.0
	D.M.	40.0	45.5	54.1	58.1	49.2
Rye hay:						
Flower	Cell.	36.3	51.8	57.4	68.2	61.9
	D.M.	37.0	50.2	53.8	59.3	55.0
Dough	Cell.	36.1	45.6	53.2	61.2	55.2
	D.M.	41.5	51.6	56.4	59.9	56.4
Seed	Cell.	32.9	43.1	49.7	59.3	55.9
	D.M.	35.5	44.5	49.1	53.6	52.7

1/ Values represent mean of 5 samples (*in vitro*) or 5 trials (*in vivo*) for each harvest date of meadow hay and 3 samples or 3 trials for each harvest stage of rye hay.

2/ R.M. and S.R.J. refer to inocula preparations, resuspended micro-organisms and strained rumen juice, respectively. Cellulose and dry matter digestion were significantly higher ($P < 0.01$) when S.R.J. was used as the inoculum than when R.M. was used.

Both cellulose and dry matter digestion were significantly higher ($P < 0.01$) when S.R.J. rather than R.M. served as the inoculum (table 1). This observation was noted with both fermentation times studied but the differences were more pronounced at 24 hours. With mixed annual range forage and solka-floc substrates, Van Dyne (1962) found higher cellulose digestion with a 48-hour fermentation period using S.R.J. inoculum than either centrifuged bacterial suspension or a phosphate buffer extract. However, when the same three inocula were used with bromegrass and orchard grass substrates, Quicke et al. (1959) found lower cellulose digestion with S.R.J. than with the other two inocula.

Variation Comparisons. In general, within-trial variation was slightly lower with 48 hours than with 24 hours fermentation and was lower with S.R.J. inoculum than with R.M. (table 2). The earlier work of Bowden and Church (1962) and more recent observations of Barnes et al. (1964) indicate a reduction in variability with increased length of fermentation periods. Van Dyne (1962) concluded that in vitro cellulose digestion results obtained with S.R.J. inoculum were as uniform as those attained by using inocula prepared by more elaborate procedures.

Table 2. Variability measurements comparing individual in vitro trials and in vivo digestion data.

Measure of variation	Measurement	In vitro fermentation period and inocula preparation 1/				Sheep digestion trial
		24 hr.		48 hr.		
		R.M.	S.R.J.	R.M.	S.R.J.	
Standard deviation	Cell.	1.77	0.75	0.91	0.64	2.74
	D.M.	1.04	0.95	1.03	1.68	2.72
Coefficient of variation, %	Cell.	3.76	1.39	1.38	0.90	4.62
	D.M.	2.28	1.83	1.74	2.66	4.97

1/ R.M. and S.R.J. refer to inoculum preparations, resuspended microorganisms and strained rumen juice, respectively.

Correlation Data. The correlation of in vitro digestion using inocula from sheep and cattle with sheep digestion was investigated by LeFevre and Kamstra (1960). They suggested that inocula from cattle and sheep could be used interchangeably if their respective rations were similar. Other workers (Bowden and Church, 1962 b, and Quicke et al., 1959) have shown a close relation between in vitro digestion, obtained with steer inocula, and conventional digestion data from sheep trials. Another problem associated with

the artificial rumen for evaluating forages is the use of inocula from an animal that receives feeds that differ from those being evaluated. Quicke et al. (1959) measured in vitro cellulose digestion of different forages when the steer providing inoculum was fed different hay rations and found that resulting data were not affected by diet of the donor animal. Later work by Van Dyne (1962) failed to confirm these results.

Correlations of in vitro trial data with sheep digestion data by forage type are shown in table 3. They are similar to those reported by other workers. Average correlation coefficients were computed for the main in vitro variables studied by using the method of LeClerg et al. (1962). These values were essentially the same: cellulose (r .94) and dry matter (r .94); 24 hours (r .95) and 48 hours (r .93) fermentation; R.M. (r .91) and S.R.J. (r .96) inocula. Slightly higher correlation was noted for meadow forage (r .96) than for rye (r .86). This was perhaps due to the limited number of rye samples studied or perhaps to differences in the diet of the steer supplying inocula and test forage.

Table 3. Correlation between in vitro digestion and sheep digestion.

		In vitro fermentation period and inocula preparation 1/			
		24 hr.		48 hr.	
Forage	Measurement	R.M.	S.R.J.	R.M.	S.R.J.
		Correlation coefficients			
Meadow hay	Cell.	.93	.98	.96	.96
	D.M.	.95	.96	.89	.94
Rye hay	Cell.	.27	.88	.79	.91
	D.M.	.80	.96	.93	.86
All samples	Cell.	.76	.91	.76	.83
	D.M.	.76	.93	.80	.83

1/ R.M. and S.R.J. refer to inocula preparations, resuspended microorganisms and strained rumen juice, respectively.

In all factors studied in this experiment S.R.J. was equal or superior to R.M. inoculum. This, along with the simplicity and ease of preparation, are indicative of the reliability and utility of S.R.J.

SUMMARY

Twenty samples of meadow hay and 9 samples of rye hay on which conventional digestibility data were obtained in sheep trials, were compared in the artificial rumen by using two methods of processing steer inoculum

(strained rumen juice - S.R.J. and resuspended microorganisms - R.M.); two fermentation periods (24 and 48 hours); and two measurements of digestibility (cellulose and dry matter). Separate in vitro trials were conducted for cellulose and dry matter at each fermentation period and with each method of inoculum preparation.

In vitro digestion obtained with 24-hour fermentation underestimated in vivo digestion, while that obtained with 48-hour fermentation overestimated animal digestion. Within-trial variation was slightly lower with 48 hour-fermentation than with 24 hours, and was generally lower with S.R.J. inoculum than with R.M. Variation from all in vitro trials was lower than that encountered in sheep digestion studies.

In vitro digestibility determinations were more closely correlated to in vivo values on meadow hay samples (r .96) than on rye samples (r .86) but correlations were not affected by length of fermentation, method of inocula preparation or digestibility measurement (cellulose or dry matter).

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