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Micro-Methods for Nutritive Evaluation Of Range Forages[1]

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In many range investigations it is desirable to obtain an index of the nutritive value of a given range plant or plant mixture. Chemical analyses for the proximate components and for other nutrients yield useful information but provide only a general indication of the nutritive value of the plant. Better estimates of the nutritive value may be obtained by conducting digestible and metabolizable nutrient trials. Frequently, however, information is required on samples of a single range plant species, part of a range plant, or plant mixture obtained by clipping or with esophageal- or rumen-fistulated animals; thus, it is impractical to secure an adequate amount with which to conduct a digestibility trial.

Considerable attention has been given during recent years to the development of methods to evaluate cellulose digestion of small samples of herbage by microtechniques. Two methods of interest for adaptation to use in range investigations in evaluating cellulose digestion are the *in vitro*-artificial rumen and the *in vivo*-nylon bag (silk bag or dacron bag) techniques. This paper presents results of preliminary studies concerning the development and modification of techniques for *in vitro* and *in vivo* nutritive evaluation of range plants.

Review Of Literature

Artificial rumen

Artifical rumen or *in vitro* procedures have been used extensively to obtain information on nutritive value of farm roughages and concentrates and to study rumen bacteria nutrient requirements in many animal husbandry investigations and are reviewed in detail by Annison and Lewis (1959) and by Barnett and Reid (1961). The artificial rumen is a system in which an attempt is made to duplicate the conditions existing in the rumen. Temperature is usually controlled at 39-40°C. by means of a water bath, anaerobic conditions are maintained by passing CO_2 gas through the test solution, acidity is controlled to near pH7 by "artificial saliva" buffer systems, bacteria obtained from the rumen are added, and a forage source is provided. Digestive activity is evaluated by manometric methods, or more commonly, by determining disappearance of a given chemical constituent during a discrete time period. Cellulose digestion is the item most commonly measured, but other factors are measured such as dry matter (Church and Peterson, 1960; Clark and Mott, 1960), gross digestible energy (Clark, 1958)[2] and digestible organic matter (Pigden and Bell, 1955) as well as digestion of other chemical constituents.

Inocula preparation methods used in *in vitro* trials vary from differentially centrifuging bacterial cells to simply expressing rumen liquor from rumen ingesta (Johnson *et al.*, 1960). Le Fevre and Kamstra (1960) indicate inocula from cattle and sheep can be used interchangeably if the rations are similar; but Hungate *et al.* (1960), Warner (1956) and Asplund *et al.* (1958) have indicated by their studies the need for obtaining the inoculum sample from the class of stock for which the evaluations are being made and from animals fed (or grazing) a diet similar to the one being evaluated. Taylor *et al.* (1960) also found closer agreement of conventionally determined and *in vitro* cellulose digestion estimates when the diet and test forage were the same rather than different forages. In addition, many other variables must be considered or held constant in *in vitro* studies including amount

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[2] *Clark, K. W. 1958. The adaptation of an artificial rumen technique to the estimation of the gross digestible energy of forages. Ph.D. Diss. Purdue Univ. 110 pp.*

of sample, particle size of the sample, method of processing the inocula, length of the fermentation period, and the type of buffer and nutrient solutions used. It is difficult to fully evaluate or reproduce the techniques used in many *in vitro* and *in vivo* studies based on procedure descriptions published in many articles. In many instances thesis and dissertation references were examined in the development of this work and some are herein cited in the footnotes.

Nylon-bag Determinations

In an attempt to overcome certain difficulties associated with *in vitro* procedures early workers developed techniques in which bags were filled with various cellulose sources and suspended in the rumen of the animal through a fistula to secure an estimate of cellulose digestion (Quinn *et al.*, 1938). The fistulated animal is comparable to the nonfistulated animal in its capacity to digest feeds (Barnett and Reid, 1961; Drori and Loosli, 1959) and thus valid inferences can be made to larger populations of animals.

Small bags of fine weave nylon, dacron, or silk varying from approximately 2″ x 4″ up to 6″ x 12″ and containing from 1 up to 50 grams of sample are suspended in the rumen for time periods varying from a few up to 96 hours (Cason, 1957[3]; Burk *et al.*, 1960; Lusk, 1961; Merrill, 1959; Tomlin, 1960[4]; and Johnson *et al.*, 1960).

Hoflund *et al.* (1948) have found a disadvantage in the silk-bag technique in that certain test materials such as paper pulp were inclined to become clumped in the bags. When the cellulose source clumped or "doughed" in a corner of the bag there was no sharp endpoint in digestion and time for the complete cellulose digestion could not be accurately determined. Erwin and Elliston (1959) reported estimates of digestion by nylon bag technique were influenced inversely by sample size and directly by length of fermentation.

Nylon bag studies (and silk and dacron bag studies) generally are used to evaluate digestibility of cellulose although dry matter digestion estimates are obtained in addition in some instances. Dry matter digestion estimates are obtained by rinsing the samples thoroughly to extrude soluble components that still may be in the bag when they are removed from the animal.

Micro-evaluation Of Individual Range Plants

Frederiksen and Washburn (1961) presented data on *in vitro* fermentation of ten hand clipped species taken from summer mountain sheep range. Wallace *et al.* (1961) presented *in vitro* digestion data on six hand clipped range plants taken at various stages of growth in two different years from sagebrush grass range. These two researches have presented valuable information on comparative *in vitro* digestibility of range plants but the approaches have limitations. The high mountain summer sheep range samples from Montana were evaluated with a rumen inocula obtained from fluid from a commercially slaughtered sheep from Colorado. The diet of the slaughtered sheep was different from the diet of sheep which graze the summer range where the plants were obtained. The Oregon sagebrush-grass range samples, obtained at different stages of growth, were digested by inocula obtained from a steer maintained on a single high quality growing ration. Nevertheless, these two studies indicate that differences in digestibiliy as affected by stage of maturity of, and differences among individual range plants are of sufficient magnitude to be evaluated comparatively by *in vitro* techniques.

Procedure-Artificial Rumen Studies

The following variables were investigated: length of fermentation period (Experiments 1 and 2); method of processing inoculum (Experiment 2); comparison of digestion of different forages to a standard cellulose source (Experiment 1); and influence of the type of forage consumed by the animal from which inoculum is obtained on *in vitro* cellulose digestion (Experiment 1 vs. 2 for two cellulose sources).

Source Of Inoculum

In all *in vitro* trials a single rumen-fistulated cow was used as the source of inoculum. This cow was fed hay *ad libitum* and had access to salt and water at all times. In the first experiment (two trials) the cow was fed a high quality alfalfa hay and in the second experiment (two trials) she was fed a low quality oat hay. After the cow had been off feed overnight inoculum was taken by opening the fistula, removing and discarding ingesta near the opening, and obtaining a sample (approximately 1½ gallons) by taking sub-samples from various parts of the rumen.

Alfalfa hay was selected as being a representative hay which would be available at many experiment stations; the low quality oat hay was selected as a hay which might approach the quality of forage being grazed on the range during the periods of the year when most western range livestock are under a nutritional stress.

Processing Of Inoculum And In Vitro Operation

In the first series of trials the

[3] *Cason, J. L. 1957 A study of digestion of cellulose and dry matter using* in vivo *and* in vitro *rumen techniques. Ph.D. Diss. Cornell Univ. 66 pp.*

[4] *Tomlin, D. C. 1960. Crystalinity of cellulose and digestibility of feedstuff cellulose in the bovine rumen. Ph.D. Diss. Univ. Florida. 86 pp.*

inoculum sample was processed by a procedure reported by Quicke *et al.* (1959) involving extracting strained rumen juice with a phosphate buffer, centrifuging the buffer extract through a Sharples super-centrifuge, and resuspending the sediment. The resuspended sediment was then used to inoculate individual tubes. The procedure of centrifuging the buffer extract of the strained rumen juice through a Sharples super-centrifuge is not easily adapted to range studies where equipment and facilities may be limited. Thus, the second experiment evaluated three different methods of preparing the inoculum: 1) rumen liquor strained through cheesecloth; 2) a phosphate buffer extract of the rumen pulp; and 3) phosphate buffer extract which had been centrifuged, plant debris discarded, and bacterial cells resuspended in phosphate buffer following, in general, the procedures of Quicke, *et al.* (1959).

Varying amounts of different cellulose sources were added to 100 ml. centrifuge tubes to provide 0.2 grams of cellulose from each source in both *in vitro* experiments. In the first experiment 12-, 24- and 48-hour fermentation periods were conducted on each of the five cellulose sources; whereas, in the second experiment only 24- and 48-hour time periods were evaluated for two cellulose sources. Duplicate tubes of each feed were digested for each time period in both experiments.

Carbon dioxide was bubbled through each tube continuously during the fermentation. Two or three drops of mineral oil were added to each tube to help prevent foaming and a few hours after initiation of fermentation and at approximately halfway through the respective fermentation periods the sides of the centrifuge tubes were washed down with the buffer solution. Blank tubes were included in each trial to determine the influence of the inocula on cellulose contents. The amount of cellulose remaining after fermentation was determined within the 100 ml. centrifuge tubes by the procedure of Crampton and Maynard (1938).

Cellulose Sources

Several types of forage samples and cellulose sources were used. These samples and their approximate cellulose contents were as follows: 1) Standard forage (an alfalfa meal forage sample furnished by Purdue University for interstate comparisons), 30 percent; 2) mature Medusahead wildrye grass *(Elymus caputmedusae)*, 32 percent; 3) immature Medusahead wildrye grass in the boot stage, 27 percent; 4) mature mixed annual range forage — primarily Wild oat *(Avena barbata)*, Soft chess *(Bromus mollis)*, Ripgut brome *(Bromus rigidus)* and Nitgrass *(Gastridium ventricosum)* 37 percent; and 5) Solka floc, 96 percent. The range forage samples were obtained by clipping, the Solka floc was obtained commercially. All samples were ground through a 40-mesh screen except the Solka floc, which was already in a finely divided form (90 percent passes through a 100-mesh screen).

Procedure-Nylon Bag Studies

The first nylon bag experiment compared mixed annual range forage cellulose digestion in rumen-fistulated cattle and sheep fed oat hay (two animals of each class of stock selected from a group of nine of each class) as affected by five fermentation times (24-72 hours), and by variations in sample weight (approximately 2-10 grams). The second nylon bag experiment was conducted by grazing these same rumen-fistulated animals on mature (July-August, 1961) mixed annual grass-forb range at the Hopland Field Station in Mendocino County in northern California. A comparison was made of 48-hour digestion of two-gram samples of two grazed forage samples (40-70 samples composited) collected a few days previously on the same range by five esophageal-fistulated cattle and seven sheep during a five-day period.

Nylon Bags And Weighting System

The nylon bags were approximately 2″ x 4″, were constructed locally from parachute cloth with approximately 120 threads per inch, and were double sewn on all seams with nylon thread. In addition to the forage sample, two glass marbles were placed in each bag to aid in mixing by preventing the sample from being "doughed" into one corner of the bag and to add weight. The tops of the bags were tied with a short length of 20-pound test braided nylon line. The "tie lines" were affixed to a main nylon "drop line" made of carpenter's cord (experiment 1) or ¼-inch three strand nylon line (experiment 2). The main drop line was attached to a ring bolt in the cap of the lucite cannula in the fistulated animals (Figure 1). Plastic ear tags were affixed to each tie line for bag identification (experiment 1) or the bags

Table 1. Cellulose digestion in 12. 24. and 48 hours *in vitro* by inoculum from a cow fed alfalfa hay.

Cellulose source	Trial 1			Trial 2		
	12	24	48	12	24	48
	(percent)					
Standard forage	34	46	51	33	43	51
Mature Medusahead wildrye	24	61	70	15	54	72
Immature Medusahead wildrye	40	68	82	31	64	79
Mixed annual range forage	31	55	67	23	51	67
Solka floc	5	72	93	5	41	89

Table 2. Cellulose digestion in 24 and 48 hours *in vitro* by inoculum obtained from a cow fed oat hay and processed in three different ways.

	Mixed annual range forage		Solka floc	
	24	48	24	48
Method of processing	———— (percent) ————			
Trial 1				
Centrifuged suspension	40	61	42	81
Phosphate buffer extract	56	73	75	92
Strained rumen juice	55	75	62	98
Trial 2				
Centrifuged suspension	38	43	41	49
Phosphate buffer extract	60	67	80	97
Strained rumen juice	58	74	77	98

were labeled directly with a special ink (experiment 2). As many as 26 bags have been suspended in both cattle and sheep with no ill effects.

A polyethylene bottle filled with lead shot was used to "sink" the bags into the ventral portion of the rumen. After 24, 36, 48, 60 and 72 hours, respectively of fermentation two bags were removed at random from the group of ten in each animal in experiment 1; a 48-hour fermentation was used for all bags in experiment 2. Duplicate bags were used for each feed or time period in these experiments.

Processing Procedure

After removal from the animals the bags were soaked in 75 percent ethyl alcohol solution to stop bacterial action, then rinsed in tap water to remove particles of ingesta adhering to the surface. The bags were rinsed as a group in an effort to minimize variations in digestion estimates due to rinse procedures. In the first experiment they were rinsed thoroughly under running tap water and then oven dried. In the second experiment they were processed in two different ways; they were rinsed as in the first experiment and half then received additional soaking and rinsing. The latter group were thrice alternately rinsed in running tap water and soaked and agitated in a large beaker of water prior to drying.

After rinsing, oven drying, and weighing each nylon bag and the sample contained was placed in a 100 ml. round bottom centrifuge tube and cellulose was determined by the procedure of Crampton and Maynard (1938). The reagents used in this procedure dissolved the nylon bags and tie lines. The plastic ear tags used to identify bags in the first experiment were removed prior to chemical analyses. Since the nylon bags were relatively inexpensive (approximately 7 cents each), it was considered better to chemically digest the bag along with the forage sample than to attempt to quantitatively remove the forage sample from the bag so that the bag might be reused.

Cellulose digestion values and dry matter digestion values both include losses of small undigested forage particles which might pass through the weave of the bag. Dry matter digestion values also include losses of soluble components from the bag during rinsing that may have not been digested.

Results-Artificial Rumen Studies

Time Of Fermentation

Tables 1 and 2 present the mean values for experiments 1 and 2. There was an increase in cellulose digestion with increasing time periods irrespective of the source of cellulose. Differences between or among time periods were highly significant in all trials except trial 2 of experiment 2. In general, these results agree with those reported in the literature with respect to increasing cellulose digestion with increasing times of fermentation for the range of time periods investigated (Quicke, *et al.*, 1959; Hershberger, *et al.*, 1959; Donefer, *et al.*, 1960).

The analysis of variance in experiment 1, following, shows very good agreement between trials conducted at different times. Experimental treatments, times of fermentation and different feeds, accounted for almost all of the variation.

Solka floc, the commercial cellulose source, appeared to be a slow starter. That is, this cellulose source had the lowest digestibility during the early stages of fermentation (12 hours), but at 48 hours it was the most digestible of all cellulose sources in all trials, independent of the method of inoculum preparation. Solka floc digestion values are comparable to many of those reported for pure cellulose sources in the literature (Hershberger *et al.*, 1959; Donefer *et al.*, 1960).

Several procedures, with respect to time of fermentation, might be used to evaluate range plants by *in vitro* techniques.

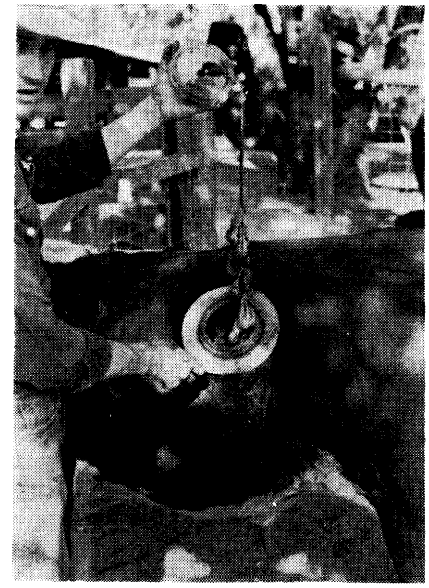

FIGURE 1. A string of nylon bags being removed after 48 hours fermentation from a rumen-fistulated steer in range digestion trials.

Single or multiple time periods of fermentation may be selected for comparisons of different samples. If there is no significant interaction between samples and time of fermentation and if the fermentation rates do not vary widely for different samples, then a single fermentation period would be more desirable as it would allow the evaluation of a greater number of samples in any given study. The results of experiments 1 and 2 would indicate that the different forage plant sources of cellulose could be comparatively evaluated for cellulose digestibility at either the 24- or the 48-hour fermentation periods (Figure 2). The rate of digestion of the different forages after 24 hours was similar with no forages changing their relative position in percent cellulose digestion between 24 and 48 hours.

Source of variation	Degrees of freedom	Mean squares Trial 1	Trial 2
Times (T)	2	5566**	6353**
Feeds (F)	4	305**	224**
T x F	8	390**	319**
Error	15	4	5

**Significant at the 1 percent level

Influence Of Cellulose Source

Solka floc had the highest cellulose digestion of all samples tested. This is to be expected as the Solka floc is approximately 96-97 percent cellulose and is free from such indigestible materials as lignin. In addition, the Solka floc is in a more finely divided form than are the ground forage samples. Most mature range plants contain approximately 25-50 percent cellulose. Lignification of bundles and fibers in the plants makes much of the cellulose unavailable to the cellulolytic microorganisms, although the material has been ground to pass through a 40-mesh screen (maximum particle size of 1 mm.) (Kamstra, 1955[5]).

The mixed annual range forage fed pelleted to wethers in a conventional digestion trial (in crates) had cellulose digestibility coefficients of aproximately 55 percent when fed with a protein supplement, and from 32-45 percent when fed as the only feed (unpublished data, W. C. Weir). This mixed annual range forage, averaged over both trials in experiment 2, had 48-hour average digestibility of approximately 52 percent by *in vitro* procedures. In general, 48-hour *in vitro* cellulose digestibility values are higher than *in vivo* values obtained by conventional trials (Le Fevre and Kamstra, 1960). Several factors influence this difference in estimates of cellulose digestion including such items as size of grind and length of fermentation.

Immature Medusahead wildrye generally had the highest cellulose digestion values of all forages at different times of digestion. It is well known that immature plants are more nutritious and more digestable than are mature plants of the same or related species (Kamstra *et al.*, 1958). Cellulose digestion of mature Medusahead wildrye was similar to that of the mature mixed annual range forage for fermentations of greater than 24 hours. This would indicate that there may be little difference in cellulose digestion among annual range grasses at maturity when the entire plant is sampled. This relationship, however, needs further and more detailed study.

Influence Of Inoculum Preparation

The data from experiment 2 (Table 2) indicate that comparable estimates of cellulose digestion in mixed annual range forage and in Solka floc were obtained by strained rumen juice or phosphate buffer extract inocula.

The following summary shows the results of the analysis of variance of experiment 2 data:

These data indicated that methods of processing the inocula were of significant importance in both trials. The magnitude of the variation due to cellulose sources was approximately the same in both trials, but because of the high degree of variability in trial 2 of this experiment, cellulose sources were not significant in both instances. The anomalous behavior of Solka floc has been referred to in a previous section.

Average percent digestibility of cellulose for the two cellulose sources in experiment 2 was approximately 75 percent for the strained rumen juice and the phosphate buffer extract, but only approximately 50 percent for the centrifuged suspension. One might expect the latter method to give higher cellulose

Source of variation	Degrees of freedom	Mean squares Trial 1	Trial 2
Cellulose source (S)	1	1537**	1755
Times (T)	1	3301**	923
Methods (M)	2	880**	3022**
S x T	1	109	63
S x M	2	39	236
T x M	2	92	77
S x T x M	2	37	5
Error	12	29	441

**Significant at the 1 percent level.

[5] *Kamstra, L. D. 1955. Digestion of cellulose from different sources by rumen microorganisms. Ph.D. Diss. Ohio State Univ. 106 pp.*

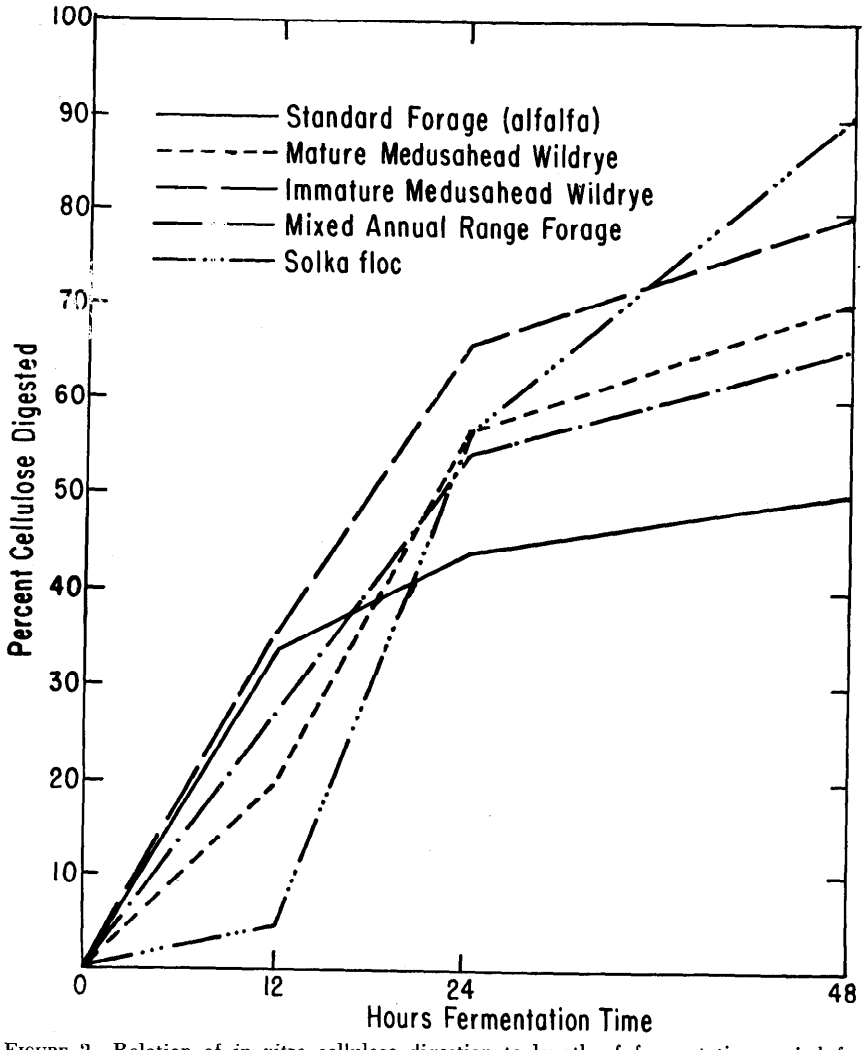

FIGURE 2. Relation of *in vitro* cellulose digestion to length of fermentation period for different cellulose sources.

digestion values as it represents a more concentrated source of cellulolytic microbes free from debris of plant material. Dehority *et al.* (1960) found the sediment of rumen fluid sedimented between 1500 x G and 3000 x G to contain the majority of the cellulolytic activity. In addition, Quicke *et al.* (1959) reported 48-hour *in vitro* cellulose digestibility values for four pasture forages using these three methods of inocula preparation and indicated slightly higher digestion values with the centrifuged and resuspended rumen microorganisms. However, their inocula was obtained from a steer fed a good quality alfalfa hay, whereas in this experiment the inocula came from a cow fed a low quality oat hay. Perhaps the differences in the diets of the fistulated animals can account for large differences in quantitative and qualitative characteristics of the rumen microorganism populations and that different types of microbes will react differently to various methods of processing.

Effects Of Base Diet

The difference in digestibility of mixed annual range forage and Solka floc at either 24 or 48 hours in experiment 1 as compared to experiment 2 is due to the influence of diet of the rumen-fistulated animal from which the inocula was obtained. In both of these experiments data are available for the centrifuge and suspension method of inoculum preparation. Procedures and donor animal were the same in these trials. Figure 3 shows a comparison of the cellulose digestion of mixed range forage and Solka floc as influenced by the source of roughage fed to the cow. There was greater cellulose digestion in both the mixed range forage and the Solka floc in the trials where the cow was fed alfalfa hay as compared to the trials where the cow was fed oat hay. The question arises as to the validity of using the inoculum from an animal fed on a different kind of feed than the one being evaluated. Further studies are in progress to evaluate this effect under range conditions.

Results—Nylon Bag Studies

Effect Of Sample Weight

The data obtained from the first nylon bag experiment are presented graphically in Figure 4. Individual animal values compose the five "sheets" in the center. Each sheet is delineated by eight bag values which show considerable variation. However, over all fermentation times percent cellulose digestion was inversely related to sample size and directly related to fermentation time (Figure 4). In order to assess the relationship between sample weight and percent cellulose digestion, regression equations were calculated showing the linear relationships between percent digestion (Y_i) and sample weight (X_i) for each class of stock at various fermentation times. Three equations of best fit were selected; two of which were highly significant and the third approaching significance. These equations were for sheep over all times and for cattle for 24-36 hours and for 48-72 hours of fermentation. Sample size was more important in decreasing nylon bag cellulose digestion of sheep than of cattle forage samples. Erwin and Elliston (1959) also report a linear decrease in digestion with increasing sample size.

A much more uniform set of data were obtained with a fair agreement between duplicates when cellulose digestion percentages were adjusted to a common sample size by the above mentioned equations. Over all classes of stock and time periods the average absolute differences between duplicates was approximately 5 percent. These duplicate values do not agree as closely as do the duplicate *in vitro* tubes but the nylon bag technique is a less expensive and more flexible procedure and a greater number of replicates could be used as compared to artificial rumen date. Some technical difficulties in the method of bag removal from the fistule added to the relatively high degree of variation between duplicates, especially from the sheep; these difficulties have been overcome in subsequent studies.

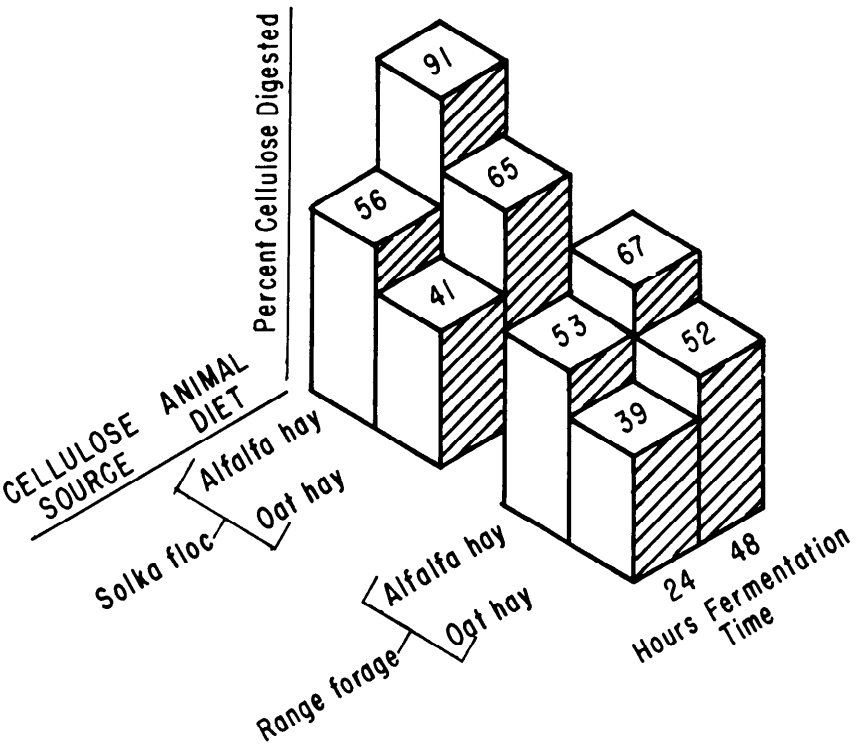

FIGURE 3. Comparison of percent cellulose digestion *in vitro* by inoculum from a cow fed alfalfa hay and from the same cow fed oat hay. Each value is the mean duplicates in each of two trials.

The adjusted values (to a 2-gram sample) of percent cellulose digestion were then subjected to an analysis of variance as follows:

Source of variation	Degrees of freedom	Mean square
Times of fermentation (T)	4	841**
Linear	1	2618**
Quadratic	1	707**
Cubic	1	14
Quartic	1	25
Animals (A)	3	168**
Cattle vs. sheep	1	2
Between sheep	1	352**
Between cattle	1	149*
T x A	12	30
Error	20	31

*Significant at the 5 percent level.
**Significant at the 1 percent level.

This analysis is only an approximation due to the fact that there were repeated measurements from the same organism over time; that is, the same animal furnished estimates of cellulose digestion over the five different time periods. Thus, the errors (E_{ijk}) are not independent, which is an assumption underlying the analysis of variance. However, it was found that although one animal (sheep #4) had the highest estimates of cellulose digestion in three of the five time periods and another (sheep #8) had the lowest estimates of cellulose digestion in three of five periods, in general, there was a good scattering of the rank of the individual animal values in the various time periods. This indicates a fair degree of independence of measurements and thus the analysis of variance was conducted.

Length Of Fermentation

Differences in cellulose digestion among lengths of fermentation periods were highly significant. Cellulose digestion increased with increasing time periods of fermentation up to 60 to 72 hours; the latter values were slightly lower than the 60-hour values (Table 3). Orthogonal polynomial coefficients were used to determine the time effects due to linear, quadratic, cubic and quartic equations. The time response analysis indicates that linear effects and quadratic effects were both important with linear effects being more influential. This is shown in the portion of Figure 4 where the values of cellulose digestion adjusted for two-gram sample size are plotted against time. The digestion curves tend to have a linear relation to time in the early fermentation periods and flatten out or drop off during the latter two fermentation periods.

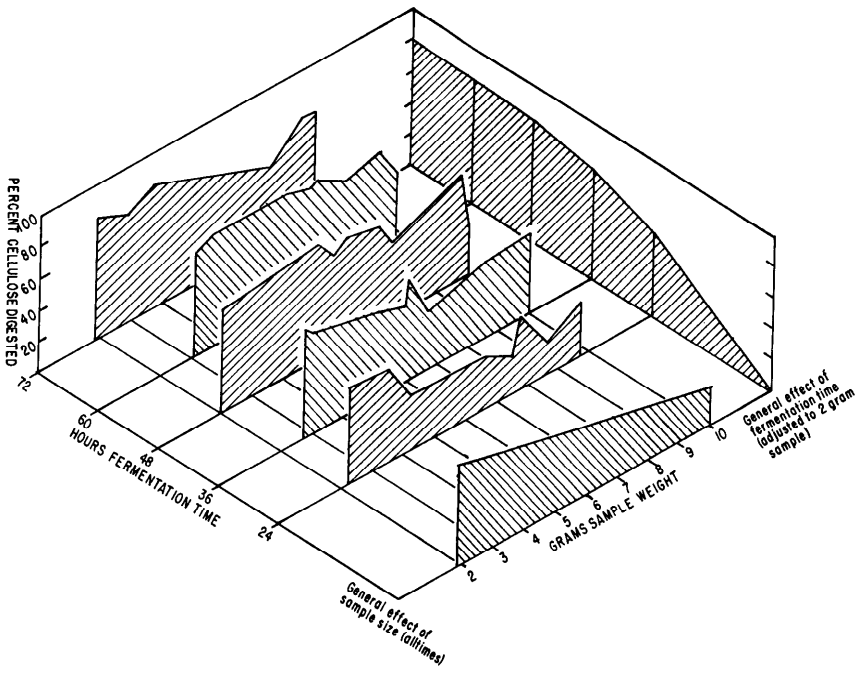

FIGURE 4. Relationship of sample weight and fermentation time to *in vivo* nylon bag cellulose digestibility. Individual animal values for 24 to 72 hours are not corrected for sample size differences.

Animal Variations

The significant effects of variation among animals may be subdivided into three groups. First, variation between cattle and sheep contributes part of the total animal variation; second, variation between sheep; and third, variation between cattle. The sum of squares for between sheep and between cattle were calculated by evaluating the variation between sums of individual animal values (within a class) over the various time periods less a "class correction factor" (the total class values squared and divided by the number of samples entering into that determination). This procedure can be checked by totaling the sum of squares due to the various animal group subdivisions which checks (within rounding-off error) with that sum of squares for variation between animals.

There was no significant difference between the estimates of cellulose digestion of this forage sample between the average cattle values and the average of sheep values when they were fed the same base feed (Table 3). However, variation between animals of the same class was highly significant for sheep and significant for cattle. Since this analysis is based on data from only two animals within a class, further study is necessary to fully evaluate relative intraspecific variation.

Times x animals interaction was not significant indicating that increasing or decreasing the length of fermentation periods did not alter the relationship between the two classes of stock. Neither were the times interactions with any of the animal subdivision categories significant and these results are thus not presented in the above analysis of variance table.

Range Digestion Trial: Cattle vs. Sheep Digestion Capacity

The mean values of percent cellulose digestion and dry matter digestion in the nylon bag test on the range are presented in Table 4. The analysis of variance of the range nylon bag trial is presented in the following summary:

Source of variation	Degrees of freedom	Mean squares: Cellulose digestion	Mean squares: Dry matter digestion
Animals (digesting) (A)	3	57**	55**
Cattle vs. sheep	1	111**	107**
Between sheep	1	6	39**
Between cattle	1	54*	19*
Forage grazed (F)	1	84**	1
A x F	3	2	1
Rinsing procedure (R)	1	117**	824**
A x R	3	2	6
F x R	1	8	23*
A x F x R	3	3	6
Error	15	8	4

*Significant at the 5% level; **significant at the 1% level.

Table 3. Cellulose digestion of mixed annual range forage by *in vivo* nylon bag technique with fistulated animals fed a low quality oat hay.

Length of fermentation	Sheep #4	Sheep #8	Cattle #33	Cattle #36
(hours)	(percent)			
24	57*	50	62	51
36	73	70	76	64
48	82	69	80	74
60	90	77	77	77
72	82	76	79	80

* Values adjusted to 2 gram sample.

Table 4. Dry matter and cellulose digestion of grazed range forage by *in vivo* nylon bag technique with fistulated animals grazing foothill annual range.

Type of forage	Rinse procedure	Cellulose Digestion Sheep #4	Sheep #8	Cattle #33	Cattle #36	Dry Matter Digestion Sheep #4	Sheep #8	Cattle #33	Cattle #36
		(percent)							
Cattle grazed	Thorough	66	66	63	61	67	65	64	62
	Light	61	60	59	56	57	53	52	48
Sheep grazed	Thorough	63	62	61	54	66	63	64	60
	Light	60	57	56	54	59	55	52	53

Two-way and three-way interactions involving animal categories and other treatments were evaluated and found to be of small magnitude and insignificant and thus are not presented in the above table. In general, the variation among animals and between classes of stock was of similar magnitude and significance for both cellulose and dry matter digestion estimates. In contrast, intraclass comparisons were more variable.

These data indicate there were also significant differences among the four animals used as "digesters" when grazed upon the range. The major variation among animals was due to highly significant differences between cattle and sheep. This is in contrast to their performances when fed oat hay wherein there were no differences between cattle and sheep. The reasons are not clear why, but on the range, sheep provided a slightly but highly significantly greater cellulose digestion of 3.7 percent. Both of the sheep had higher average cellulose digestion values as determined by the nylon bag technique than did the cattle. The differences in cellulose digestion between sheep were small and nonsignificant (1.2 percent); whereas, the differences between cattle (3.7 percent), although comparatively small, were highly significant.

In the range trials sheep digested 3.7 percent more dry matter of range forage than did cattle (highly significant). The animals maintained their "relative ranks" in digestion of cellulose and dry matter; these were in decreasing order—wether #4, wether #8, steer #33 and steer #36. This ranking was consistent over both forage sources on the range but not when these animals were fed low quality hay. This indicates that individual animal grazing habits are important in range digestion trials although there was a greater difference between highest and lowest "cellulose digesters" in the hay feeding trial as compared to the range grazing trial, respectively 8.4 percent and 6.1 percent.

It was observed that the sheep were more selective in eating the oat hay than were the cattle, but evidently, there was a much greater difference in the diets of the two classes of stock when they were allowed to graze on the range. Average chemical composition in percent for the diet of esophageal-fistulated cattle and sheep, respectively, during this grazing trial was: crude protein, 6.3 and 7.5; ether extract, 1.4 and 1.5; lignin, 11.9 and 12.1; cellulose, 37.5 and 35.3; and gross energy, 4850 and 4350 calories per gram. A higher quality diet results in a more dense and vigorous microflora as compared to a low quality diet (Annison and Lewis, 1959). The result of a larger and more vigorous population of rumen microorganisms is reflected in increased cellulose digestion as was indicated in the *in vitro* investigations comparing a diet of alfalfa hay with oat hay (Figure 3). These data illustrate the importance of using the class of stock on the range for which digestion estimates of various forages are desired.

Digestion Of Cattle vs. Sheep Grazed Forage

Cattle grazed forage was higher in cellulose digestibility (61.5 vs. 58.2, highly significant difference) but similar to sheep forage in dry matter digestibility (58.5 vs. 58.9, nonsignificant difference). There was no significant interaction of animals with forages indicating that sheep could digest cellulose and dry matter in cattle grazed forage as well as could cattle and that cattle could digest the sheep grazed forage as well as could sheep (Table 4).

The relative similarity of cattle and sheep diets as measured by chemical composition, especially lignin and cellulose content, would lead one to expect similar digestibility. Herbage supply was at all times adequate to the animals during this grazing period, approximately 1150 pounds per acre of total grass and forbs, thus allowing the animals considerable selectivity in their grazing. Thus, the differences in cellulose digestibility of the two forages may be related to differences in botanical rather than chemical composition of the diets of the cattle and sheep. Further studies are in progress to evaluate comparative botanical composition of range cattle and sheep diets.

Rinse Procedure Effect

Differences between average percent cellulose digested for the two rinse procedures were highly significant although relatively small, 3.8 percent (Table 4). This indicates that comparatively little more cellulose is removed by the exhaustive rinsing procedure as compared to a light rinse. Rinse procedure did not interact significantly with forages nor with animals for cellulose digestion.

There was greater than 10 percent difference (highly significant) in estimates of dry matter digestion by the two rinse procedures. This would be expected as considerable color was extruded from the bags during the rinse procedure. Further rinsing on a few bags (not reported herein) indicated that essentially all of the soluble material had been removed from the bags by this degree of rinsing. There was essentially no difference, 0.4 percent, between percent dry matter digestion estimates of cattle forage and sheep forage.

Although all the bags were rinsed at one time by one individual it is difficult to say that they each had an equal amount of rinsing. Thus, it is felt that the estimate of dry matter digestion is a much less accurate measure than is the estimate of cellulose digestion which does not depend as much on a qualitative procedure such as rinsing the bags.

The interaction between forage sources and rinse procedures was significant. There was approximately 11.8 percent difference in dry matter digestion of the thoroughly versus lightly rinsed cattle forage samples as compared to a difference of only 8.4 percent between the thoroughly rinsed versus lightly rinsed sheep forage samples. This effect is not readily explainable. In both instances the thoroughly rinsed samples yielded higher estimates of dry matter digestion than did the lightly rinsed samples.

In Vivo vs. *In Vitro*

Figure 5 presents mixed annual range forage cellulose digestion values extracted from the second *in vitro* and the first *in vivo* experiments. In these experiments the rumen-fistulated animals were fed a low quality oat hay. When averaged over all samples there is a remarkably good agreement between nylon bag and artificial rumen (with strained rumen juice) cellulose digestion at both 24 and 48 hours. Cason (1957) did not find such good agreement between silk bag and artificial rumen cellulose digestion but Merrill *et al.* (1961) and Lusk (1961) in studying grass and alflafa hays found reasonably good agreement between nylon bag and artificial rumen cellulose digestion estimates.

Summary and Conclusions

The results of preliminary investigations on variables affecting estimates of cellulose digestibility and dry matter digestibility by artificial rumen and by nylon bag techniques are presented. Studies with both cattle and sheep under corral feeding and range grazing conditions were conducted. Forage sources included hand clipped range plants and forage samples taken from esophageal-fistulated steers and wethers grazing the same range as the rumen-fistulated

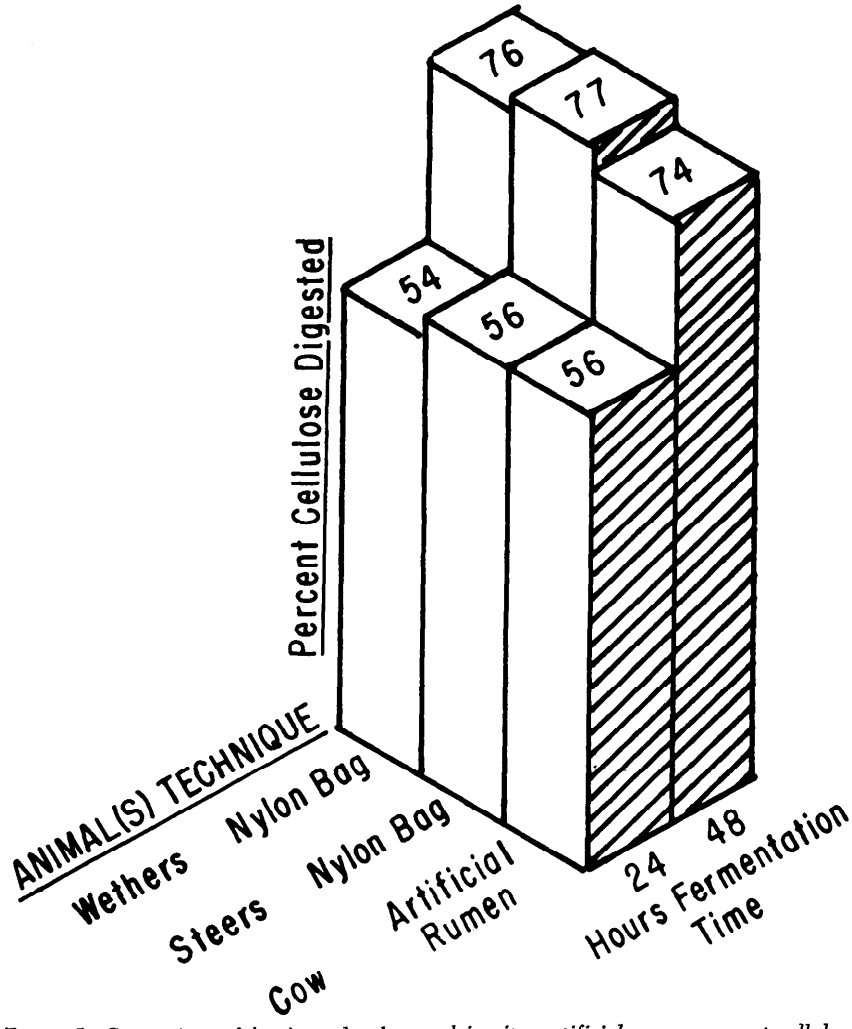

FIGURE 5. Comparison of *in vivo* nylon bag and *in vitro* artificial rumen percent cellulose digestion of mixed annual range forage when fistulated animals were fed a low quality oat hay.

animals which contained the nylon bags.

Using a constant size and manner of processing and analyzing samples, but with different cellulose sources, the following variables were studied in artificial rumen investigations: time of fermentation period, method of preparing inocula, and effects of diet of the fistulated animal from which the inocula was obtained.

It was found that there were increasing *in vitro* cellulose digestion values with time for all cellulose sources investigated over the time periods studied. From 24 to 48 hours all samples of forage maintained their relative digestion values. Inocula prepared by simply straining rumen contents through several layers of cheesecloth gave as high and as uniform cellulose digestion values as did more elaborate procedures of processing the inocula. The diet of the fistulated animal influenced the estimates of cellulose digestion. Cellulose digestion values of range forage and pure cellulose samples were considerably higher when the base diet was alfalfa hay than when the base diet was oat hay.

Using the same size and weave of nylon bag, animals, the chemical procedures, the following variables were studied in nylon bag investigations: effect of sample weight, length of the fermentation period, variations between cattle and sheep, animal to animal variation in the same class of stock, and different procedures of rinsing the bags after removal from the animals.

Sample size was inversely related to *in vivo* nylon bag cellulose digestion in the range of sample sizes investigated (from two to 10 grams of ground mixed annual range forage) in both cattle and sheep. Length of fermentation period appeared to have a linear and quadratic relationship to cellulose digestion in the time periods evaluated (24-72 hours) for both cattle and sheep. Significant differences were found between cellulose digestion of forage samples between rumen-fistulated cattle and sheep when they grazed together on the range but not when they were fed hay. Differences in cellulose and dry matter digestion between animals of the same class of stock were variable for cattle, sheep, and type of diet. Simple rinsing of nylon bags upon removal from the animal as compared to thorough and repeated soaking, agitation and rinsing of the bag resulted in highly significant but small differences in estimates of cellulose digestion but in large differences (highly significant) in estimates of dry matter digestion with the light rinse values being the lower. Cellulose digestion in cattle-grazed forage was highly significantly greater than cellulose digestion in sheep-grazed forage when tested by both classes of stock, but no signficant differences were found in dry matter digestibility by these techniques between these two forages. Sheep were better able than cattle to digest cellulose and dry matter in both cattle and sheep forage samples when grazed on the same range with the cattle as measured by nylon bag technique.

Similar estimates of cellulose digestibility in a given feed were found in these preliminary artificial rumen and nylon bag studies. There were closer agreements between artificial rumen duplicate tubes than between duplicate nylon bags.

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