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Title A COMPARISON OF IN VIVO AND IN VITRO TECHNIQUES FOR THE EVALUATION OF VARYING ROUGHAGE CONCENTRATE RATIONS

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The objective of this study was to compare the total collection (in vivo), nylon bag, and in vitro digestibility techniques under standardized conditions. Twin steers were used simultaneously to measure the digestibility of five rations consisting of the following ratios of alfalfa hay to steamed roller barley: 4:0 (I), 3:1 (II), 2:2 (III), 1:3 (IV), and 0:4 (V). One trial was completed in the in vivo study using a six day collection period, whereas two trials were run for each of the other two techniques. Fermentation periods of 24, 48, and 72 hours or 12 and 24 hours were used for the nylon bag and in vitro studies, respectively.

In vivo energy, crude protein, ether extract, and dry matter digestibility and TDN increased as the roughage:concentrate decreased from 4:0 to 0:4. The inverse relationship was noted for cellulose and crude fiber digestibility.

The variability in dry matter digestibility, as measured by standard deviations and coefficients of variation, was highest in the nylon bag and lowest in the *in vivo* procedure. The difference between trials, using dry matter digestibility as the criterion, was generally non-significant for both the nylon bag and *in vitro* techniques. It was concluded that repeatability between trials is not a factor when using these techniques if sufficient replications are used.

Differences between animals within rations were greater than expected; however, when the data were pooled across all rations, the steers were not significantly different.

The rate and variability of nylon bag and *in vitro* dry matter and cellulose digestion was greatest in the first digestion period in each of the five rations. However, as the rations included concentrates, the rate of nylon bag dry matter and cellulose digestion decreased in the same period.

A comparison of the three techniques, using the mean dry matter and cellulose digestion coefficients, showed the *in vivo* dry matter digestion to compare closely with the 48 hour nylon bag and 24 hour *in vitro* digestion. The nylon bag and *in vitro* cellulose digestibility underestimated the *in vivo* digestion of cellulose.

The nylon bag dry matter digestibility at 48 and 72 hour fermentation periods and *in vitro* dry matter digestibility at 12 and

24 hours was significantly correlated with the *in vivo* digestibility of ether extract, energy, dry matter, cellulose, and crude fiber. Similar correlations were obtained when nylon bag and *in vitro* cellulose digestion was correlated with *in vivo* digestibility of the chemical components. Correlation coefficients between the nylon bag and *in vitro* techniques, using dry matter and cellulose digestion as criteria, showed a close relationship at the longer digestion periods. Regression equations developed from these correlations showed no significant ($P<.01$) difference between the predicted and actual *in vivo* dry matter digestibility of eight substrates when using equations developed from the nylon bag and *in vitro* dry matter digestibility in this study.

The effect of inoculum source on substrate digestion was studied using the *in vitro* technique. The data showed that all-roughage and all-concentrate substrates are digested more completely with inoculum from animals maintained on the same diet. However, when measuring the digestibility of a mixed substrate (roughage and concentrate), the most accurate results, as measured by a standard, were obtained when using an inoculum from donor animals maintained on a mixed diet, regardless of the proportion of roughage and concentrates.

A COMPARISON OF IN VIVO AND IN VITRO TECHNIQUES FOR THE EVALUATION OF VARYING ROUGHAGE-CONCENTRATE RATIONS

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

A COMPARISON OF *IN VIVO* AND *IN VITRO* TECHNIQUES FOR THE EVALUATION OF VARYING ROUGHAGE-CONCENTRATE RATIONS

INTRODUCTION

Assessing the nutritive value of feedstuffs has presented a constant challenge to nutritionists for over a century. The Weende scheme of analysis, conceived by Hennenberg and Stohmann in 1864, has evolved through modifications by Atwater (1894), Hills (1900), Woll and Humphrey (1910) and Haecker (1914) into the "total digestible nutrient" (TDN) system. Henry and Morrison adopted the system in 1915 and through 12 editions of their "Feeds and Feeding" have firmly established the system as the standard for feedstuff evaluation and ration formulation in the United States. The more refined metabolizable and net energy systems are much preferred as precise measures of nutritive value. The TDN system will, however, most likely be used for many years since a great number of values are available for a multitude of feedstuffs.

In recent years a trend has developed to find a single entity in the chemical make-up of a feedstuff that would precisely predict its nutritive value for the live animal. This work has been primarily concerned with forages and low grade concentrates since the TDN system tends to over-estimate their productive value in ruminants. Thus, the need for newer and improved methods for digestibility

determinations has arisen since these chemical entities being studied are generally a function of the digestibility of the feedstuff.

The total collection technique (in vivo) has been routinely employed in determining the apparent digestibility of nutrients constituting the TDN system. This technique is accurate and dependable when conducted under carefully controlled conditions. However, it requires a 20 to 30 day preliminary period followed by a six to ten day collection period. An exact accounting of all feed consumed and feces voided during the collection period must be made. The excreta must be weighed periodically and representative samples taken for chemical analyses. Care must be taken to prevent contamination of the feces with urine or other materials that might be reflected in the analysis of one or more of the constituents. As in any biological study, the animals themselves present one of the greatest sources of error. One animal and one trial is not adequate in determining the digestibility of a feedstuff. In the past, monozygotic twins have been used to reduce the number of animals required, but still there are limitations when considering the feed required for each trial. In many instances, digestibility trials are prohibitive when only a small sample of a particular feedstuff is available or when labor and expenditures are limited.

Studies in the area of rumen digestion have advanced rapidly with the advent of the permanent rumen fistulation technique. This

technique has been a forerunner in the development of several new procedures which show considerable promise in determining the digestibilities of feedstuffs. Two of these methods are the bag and artificial rumen (in vitro) techniques.

The bag technique consists simply of suspending bags constructed from dacron, silk, or nylon, containing a weighed substrate, into the rumen of a fistulated animal and allowing it to remain for various lengths of time. The disappearance of material from the bag is considered to be the digestible portion of the feedstuff. The efficacy of this technique depends of course on its relative value when compared to the same data collected using the total collection technique.

The other technique receiving increasing interest is the artificial rumen or in vitro procedure. It involves incubating a substrate in vitro with diluted rumen fluid. The in vitro technique requires only the maintenance of a readily available source of rumen fluid, a small amount of inexpensive equipment and a limited supply of the test diet. This procedure is quite simple as indicated by Walker (1959) after studying a number of procedures. He stated: "It was found that complexity was not a criterion for obtaining digestibility values which were in close agreement with in vivo determinations".

The artificial rumen and nylon bag have been used principally

in determining cellulose and dry matter digestion of forages. Only a limited supply of information is available for either technique when used with high concentrate rations. With the increased emphasis on high concentrate feeding in ruminants, there seems to be some merit in determining the feasibility of using these techniques to evaluate the nutritive value of rations containing substantial amounts of concentrates.

This study herein was designed to evaluate the nylon bag and in vitro techniques with varying roughage-concentrate ratios and to compare these data with those determined using the conventional digestion trial. The standardization of environmental conditions, source of inoculum, length of fermentation period and species of animal was necessary to obtain a valid comparison.

REVIEW OF LITERATURE

Digestion in Ruminants

In this review of literature no attempt has been made to review all areas associated with the complex mechanisms and interactions of ruminant digestion. However, mention is made of some of the more important areas. A comprehensive symposium on the physiology of digestion in the ruminant has been compiled into book form by Dougherty and associates (1965). Earlier reviews by Edwards (1955) and Huffman (1953) present excellent pictures of the interactions between the digested material and rumen microbes.

Bacteriology of the rumen has not received its due consideration with respect to specific function since the numbers of organisms are tremendous and their diverse nature discourages quantitative studies. The situation is further complicated by the shifting population as a result of changes in the diet. Most attempts to classify bacteria have generally been in the area of their function in attacking particular substrates or producing certain end-products.

Rumen protozoa have been classified into over thirty genera consisting of two main categories, holotrichs and oligotrichs (Clarke, 1964). The holotrichs seldom ingest plant material but are concerned primarily in the utilization of readily available carbohydrates. They appear at present not to have any symbiotic

relationship with the bacteria. Consequently, they are fairly easy to isolate and can be raised in the absence of bacteria. The oligotrichs, on the other hand, appear to be partially dependent on the bacteria for their function and when separated from them do not live for any length of time.

Protein digestion in the rumen is dependent on microbial action with the primary products being ammonia and volatile fatty acids. Bryant (1964) has demonstrated that 82 percent of the isolated strains of bacteria require ammonia as their principle source of nitrogen. These microbes use ammonia, urea, purines, and pyrimidines to construct amino acids and bacterial protein that is later digested in the lower tract by proteolytic enzymes.

Carbohydrate metabolism in the rumen is the principle source of energy to both microbes and host. Carbohydrates in feedstuffs are first hydrolyzed to simple sugars and further degraded by rumen microorganisms into volatile fatty acids (VFA). Some monosaccharides are used in the construction of microbial polysaccharides which subsequently are redigested and absorbed in the lower gut. It appears that the Embden-Meyerhof glycolytic pathway is the major route of hexose fermentation by rumen microbes, although the hexosemonophosphate pathway has also been shown to function (Wood et al. 1958). Fibrous carbohydrates are fermented

slowly due to their insolubility and association with lignin.

Lipid digestion in the rumen has received considerable attention in recent years. These studies have indicated that dietary fat is not made up principally of triglycerides containing long chain fatty acids as once thought. This theory has given way to the findings that most lipids consist of galactosyl, glyceryl esters of fatty acids and that the triglyceride fatty acids represent only about three percent of the total fatty acids (Garton, 1960).

Hydrogenation of unsaturated fats is one of the more important aspects of lipid metabolism in the rumen. This has been demonstrated in recent years and suspected for a long time since depot fat in simple stomached animals contain appreciable amounts of unsaturated fatty acids.

The chemical makeup of a feed can have a decided influence on its apparent digestibility. Sineschchekov (1965) has indicated that feedstuffs rich in cellulose require considerable processing in the mouth as an initial part of digestion. He states that assimilation rate can be one and one-half to two times higher if some of the roughage is replaced by succulent feeds.

The author is cognizant of the fact that many factors affect the digestibility of roughages. Data are available indicating that fiber content, forage intake, protein content of the forage, energy of the ration, particle size and mineral content can alter the

digestibility of forages. Van Soest (1965) indicated that the chemical composition of a feed is much more closely related to digestibility than voluntary intake. His work suggested that as the cell-wall constituents increase, voluntary intake declines with an increasingly negative slope. These data are consistent with the theory that fiber mass restricts intake and that digestion proceeds according to the rate at which these constituents can be broken down and passed into the intestinal tract.

Moir and Harris (1962) have shown that when the nitrogen intake falls below 0.74 percent of the total feed intake, there is a decrease in the digestibility of the dry matter and crude fiber. Additional nitrogen added above 0.96 percent of the total feed intake did not appreciably increase the digestion of dry matter or crude fiber.

Sineschchekov (1965) suggested that in protein deficient diets, digestive glands secrete much protein, which often results in the loss of endogenous nitrogen from the animal. Stone and Fontenot (1964) studied the effect of energy levels on the utilization of nitrogen. They reported no significant effect on nitrogen retention as a result of increasing either the digestible or metabolizable energy concentration.

Total Collection Technique

The conventional method for determining digestibilities of feedstuffs for ruminants is the total collection technique (in vivo). This method involves analyzing chemically both the feed and feces for nutrient components. The portion of the nutrient not accounted for in the feces is termed the apparent digestibility of that nutrient. The percentage of each nutrient digested is called the digestion coefficient for that nutrient.

Determining the digestibility of a feed involves a preliminary period in which the animal is fed the ration for a few days, so that all residues of former feeds may pass from the digestive tract. The question has often been raised as to the length of the preliminary feeding period. Nicholson et al. (1956) used preliminary periods ranging up to 44 days with rations varying widely from roughage to concentrates and concluded that the optimum preliminary period lies between 16 and 30 days. Lloyd, Peckham, and Crampton (1956) noted in sheep a rhythmic fluctuation in the digestibility of dry matter, crude protein, crude fiber, ether extract and nitrogen free extract of both grain and roughage rations when extending the preliminary period up to as long as 60 days. They concluded that there seemed to be no justification for longer preliminary periods than ten days.

The length of collection period has been investigated by several workers. Staples and Dinusson (1951) compared the relative efficiency of seven and ten day collection periods and observed a difference in efficiency between the two of less than two percent when using the digestibility as the criterion. Clanton (1961) also has compared seven and ten day collection periods and noted, with the exception of crude protein and crude fiber, that digestibility was less variable with coefficients calculated from ten day collection periods. Hale et al. (1963) found five day collection periods to be adequate when using steers.

Total collection trials necessitate the use of digestion stalls or fecal collection bags with provisions to prevent the contamination of feces with urine. Several workers (Briggs and Gallup, 1949; Horn, Ray, and Neumann, 1954; Nelson et al., 1954; and Erwin et al., 1956) have designed digestion stalls for use in total collection trials with steers. Hobbs, Hansard, and Barrick (1950) developed a stall for the separation of feces from urine when using heifers.

Methods for preserving and handling feces are of great importance in obtaining accurate results. Lindahl (1959) reported satisfactory results when drying sheep feces at 60°C in a forced air oven. Jacobson, Kane, and Flatt (1959) canned bovine feces and stored them at room temperature. He found no differences between this method and conventional methods of freezing, although the

canning of feces proved to be an efficient and simple means of preserving fecal samples. Other workers have found satisfactory results by simply drying samples at 50-60°C and storing at room temperature in screw-top jars until chemical analyses could be completed (Alpan, 1965; and Smith, 1963).

Nylon Bag Technique

In vivo digestion trials are prohibitive for screening large number of forages or feedstuffs because of the time and expense involved. An accurate, simple and rapid method used in the evaluation of feedstuffs is the nylon bag technique.

Types of bags used for these studies have certainly not been standardized. Van Dyne (1962) used two x four inch nylon bags with approximately 120 threads per inch; Belasco, Gribbins, and Kolterman (1958) experimented with dacron bags that were five x seven inches and from cloth of approximately 100 threads per inch; Erwin and Elliston (1959) used four x eight inch nylon bags; and Yang, Ingalls,and Thomas (1962) used bags two x five inches made from nylon cloth with 64 threads per inch. Hopson, Johnson, and Dehority (1961) used dacron bags three x one and one-half inches constructed from 100-mesh dacron cloth and Ralston, Church,and Oldfield (1962) and Miller (1963) used nylon bags three and one-half x three inches constructed from nylon material with approximately

96 threads per inch.

The position of the bags in the rumen appears to affect the digestibility of the bag's contents. Two lines of thought exist as to the importance of the position. One suggests that the bags should be held in the ventral portion of the rumen in order to insure uniform digestion. The other line of reasoning suggests the bags should be left loose, thus allowing maximum movement throughout the ingesta and simulating actual movements of feed through the rumen. Erwin and Elliston (1959) attached bags to weighted chains constructed from tygon tubing and fitted with a lead-filled pipe (one and one-half x one and one-half inch) molded in plastic. Hopson, Johnson, and Dehority (1961) tied identifying metal tags to their bags and then thrust them to the ventral portion of the rumen. They indicated that the bags retained their relative position without necessity of weights. Van Dyne (1962) placed glass marbles in his bags to insure adequate mixing within the bag and to prevent the material from becoming "doughed" in one corner. The bags were sunk to the bottom of the rumen by means of a polyethylene bottle filled with lead shot.

Other workers have allowed their bags to have maximum movement and suggested that this is a more accurate estimation of the actual digestibility than when the bags are suspended in one location (Ralston, Church, and Oldfield, 1962; and Miller, 1963).

Another inherent problem in bag studies is the leaching out of material from the bag during digestion. Hopson, Johnson, and Dehority (1963) attempted to determine what percent of the material was lost. They ground hay samples through a large laboratory Wiley mill with a screen of 72 two mm. holes per square inch. Any particles failing to pass a sieve with holes 0.05 inch in diameter were reground through the mill and mixed thoroughly with the other fraction. They then placed the bags in running water for 24 hours and found only one percent of the material was actually lost by sifting from the bags. Miller (1963) did an extensive study on this problem by suspending six bags from a reciprocating bar into a water bath for 24, 48, and 72 hour periods and found the dry matter loss was 27.9, 30.5, and 29.0 percent for 24, 48, and 72 hours, respectively. His work indicated that between 24 and 48 hours the majority of the soluble and/or fine material had been removed from the sample and that bacterial action accounted for 29, 42, and 48 percent dry matter loss at 24, 48, and 72 hour fermentation times, respectively.

The procedure used in washing the bags after removal from the rumen has been shown to have an effect on the digestibility of the substrate. Yang, Ingalls, and Thomas (1962) compared two procedures of handling bags after removal from the rumen and indicated the dry matter digestion was always less when the bags

were rinsed with water after removal than when the bags were squeezed and washed. Van Dyne (1962) rinsed his bags as a group in an attempt to minimize variations in digestion estimates. He further divided the bags into two treatments to study the effect of rinsing excessively on digestion. The first group of bags was rinsed twice under tap water while the second group was rinsed three times in running tap water and soaked and agitated in a large beaker of water prior to drying. He found a highly significant, but small, difference (3.8 percent) between the two rinsing procedures when using cellulose digestion as a criterion. However, for dry matter there was a ten percent difference between the two methods. Longer rinsing on a few bags indicated that essentially all soluble material could be removed from the bags.

The effect of ration on nylon bag substrate digestion has been considered by several workers. Van Keuren and Heinemann (1962) found that the dietary regime of the animal appeared to influence digestibility of the forage samples. Yang, Ingalls, and Thomas (1962) fed one cow alfalfa silage and another alfalfa hay. The dry matter digestion for silage or hay, in hay fed animals, was higher than in silage fed animals but did not approach statistical significance.

Hale et al. (1963) fed fistulated steers a ground alfalfa hay ration or a ration containing 60 percent of either barley or milo.

The disappearance of dry matter of milo or barley from nylon bags in fistulated steers on barley or hay rations was not affected by the ration fed; however, barley dry matter disappeared at a significantly faster rate.

Animal variation is a characteristic of any biological study and in this regard Van Dyne (1962) found significant ($P < .05$) differences between unrelated animals using either sheep or cattle; his numbers, however, were too small (only two per class) to fully evaluate relative intraspecific variation. Miller (1963) used twins (assumed to be monozygotic) and found no significant difference between the two animals.

Artificial Rumen (*In Vitro*) Technique

In recent years considerable emphasis has been placed on developing techniques by which conditions found in the rumen could be standardized and duplicated within the laboratory. These efforts have led to development and use of the artificial rumen or the *in vitro* technique.

The general procedure for *in vitro* studies involves obtaining rumen liquor (fluid containing microorganisms from the rumen) from animals maintained on rations similar to the substrate to be digested. The liquor is added to a buffer solution prepared to simulate chemically the animal's saliva. The solution is maintained

in digestion bottles at 39°C (actual temperature of the rumen) and carbon dioxide is bubbled through the contents to maintain anaerobic conditions.

The artificial rumen was developed in 1940 when Trautmann and Asher studied cellulose digestion of straw. Pearson and Smith (1943) used the method while studying the utilization of urea in the bovine rumen. Since then, scores of papers have been published with almost every conceivable approach to improving the technique.

Artificial rumen systems have been reviewed and categorized into three types which include 1) the all glass system, 2) the semi-permeable, and 3) the continuous flow apparatus developed for studies of longer duration (Smith, 1963). El-Shazly, Dehority, and Johnson (1960) compared the three and found no major differences between them; however, the all-glass appears to be advantageous because of its simplicity and rapidity.

The greatest error involved in the use of artificial rumen systems probably lies in the area of preparing whole rumen fluid as a source of inoculum. Bentley et al. (1954) studied factors needed by rumen microorganisms for cellulose digestion in vitro. Microorganisms were separated from the rumen juice by centrifugation and used as inoculum. The addition of autoclaved rumen juice and extracts of various plant materials markedly increased the rate of cellulose digestion. Johnson, Dehority, and Bentley

(1957, 1958) developed an improved inoculum by discarding the first extraction of the rumen contents, resuspending the pressed pulp in buffer, and expressing this liquor as the inoculum source. Inoculum prepared from this liquor gave higher cellulose digestion and less variation between experiments. Quicke et al. (1959) used either strained rumen juice, a phosphate buffer extract of pressed rumen contents, or resuspended ruminal microorganisms as inoculums, but noted very little difference in cellulose digestion between the three methods. Bowden (1961), Smith (1963), and Wallace, Rumburg, and Raleigh (1965) used strained rumen juice in their work and reported excellent results in terms of repeatability between runs.

The pH of the in vitro system is regulated by buffer solutions added to each digestion flask. Most buffer solutions are derivations of McDougall's preparation (1948). McDougall, in perfecting the original solution, attempted to simulate the chemical constituents of animal saliva.

To insure anaerobic conditions and constant mixing of the contents, carbon dioxide (CO_2) has been bubbled continuously through the rumen apparatus. (Cheng, Hall, and Burroughs, 1955; Belasco, Gribbins, and Kolterman, 1958; Smith, 1963; and others). However, other workers have recently indicated that this procedure is not absolutely necessary and suggest that saturation of the air in each

flask with CO_2 will yield the same results. The procedure used is simply to flush the container with CO_2 at the beginning of each trial. (Baumgardt, Taylor, and Cason, 1959, 1962; Church, 1965; and Bedell, 1966).

Walker (1959) studied the concentration and quantity of fluid needed in each digestion flask for an effective system. He reported that the volume of rumen juice did not have any affect on dry matter digestibility in his system. In earlier work, Huhtanen and Elliott (1956) had diluted samples to one-fifth of the original concentration and found no difference, although further dilutions resulted in decreased activity. Church and Petersen (1960) however, noted a linear increase in dry matter digestion in vitro as the volume of whole rumen fluid increased from 20 to 120 ml. in a system with a total volume of about 750 ml. Smith (1963) reported a statistically significant ($P<.01$) difference when the rumen liquor was increased from 5 to 15 ml. Dry matter digestibility was highest when using the 15 ml. volume.

Comparisons of Nylon Bag, In Vitro and In Vivo Techniques

The original objective in developing the nylon bag and in vitro techniques was to provide a simple and rapid method for determining the digestibilities of feedstuffs. To be of value these results must

agree with or be consistently relative to digestion coefficients obtained from conventional digestion trials.

A number of workers have compared the nylon bag and total collection technique and reported close relationships (Lusk, Browning, and Miles, 1962; Yang, Ingalls, and Thomas, 1962; and Hale et al., 1963).

Comparisons of in vitro and nylon bag data were made by Van Dyne (1962). He reported remarkable agreement between the nylon bag and in vitro technique for cellulose digestion at both 24 and 48 hour digestion periods. Belasco, Gribbins, and Kolterman (1958) had reported similar comparisons in earlier work.

More intensive work has been done in comparing in vitro data to total collection data. A number of workers have reported high correlations between in vitro cellulose digestibility and in vivo dry matter, cellulose and energy digestibility on a number of forages, principally grasses (Hershberger et al., 1959; Reid et al., 1959; Donefer, Crampton, and Lloyd, 1959; Lefevre and Kamstra, 1960; Baumgardt, Taylor, and Cason, 1962; Bowden and Church, 1962; Smith, 1963; and Wallace, Rumburg, and Raleigh, 1965).

Reid et al., (1960) reported close agreements between in vitro data on 124 forages and both total collection and dacron bag data. Hopson, Johnson, and Dehority (1963) reported similar results using alfalfa hay as their substrate.

EXPERIMENTAL PROCEDURE

The literature reviewed show that both the *in vitro* and nylon bag techniques are useful for estimating digestibility of forages. However, there is some doubt as to their efficacy if used interchangeably because of the lack of standardization in procedures when making these comparisons. Furthermore, there is only limited data pertaining to the use of the nylon bag and *in vitro* techniques with concentrates.

The objectives of this study were: 1) to standardize the conditions under which the total collection, nylon bag, and *in vitro* techniques were compared; 2) to standardize the feed and inoculum source; 3) to evaluate each technique with varying roughage-concentrate ratios; and 4) to compare varying lengths of nylon bag incubation and *in vitro* fermentation periods with *in vivo* digestion data.

General Experimental Procedure

Twin four-year old steers fitted with permanent rumen fistulas were used in these experiments. Their weight gains, pH of rumen ingesta, digestibility of rations and fractional parts thereof have in the past experiments been very similar. The steers were designated as A and B and are thus referred to throughout the text.

Steers A and B, weighing 1437 and 1458 pounds, respectively, at the beginning of the experiment, were maintained in a common lot and treated the same throughout the entire experiment.

Five rations varying in roughage-concentrate ratios were used. Rations were varied at 25 percent increments beginning with a 100 percent roughage diet as follows:

Ration	Roughage, Level	Concentrate, Level	Ratios
	%	%	
I	100	0	4:0
II	75	25	3:1
III	50	50	2:2
IV	25	75	1:3
V	0	100	0:4

Steamed rolled barley was used as the concentrate source and and first cutting chopped alfalfa hay served as the roughage source. The hay was stored loose in a bin to prevent spoilage and wastage during feeding. The chemical composition of the rations are listed in Table 1.

Table 1. Chemical Composition of the Rations Used in This Study, in Percent.

	Rations				
	I	II	III	IV	V
Crude Protein	14.18	13.41	12.66	11.91	11.16
Ether Extract	1.60	1.75	1.90	2.16	2.18
Crude Fiber	36.33	28.93	21.53	14.44	6.74
Ash	8.90	7.80	6.70	5.60	4.49
NFE[1]	29.49	38.36	46.61	56.20	65.01
Cellulose	32.74	25.93	17.68	9.47	3.60
Dry Matter	90.50	90.25	89.40	90.20	89.58
Energy, KCal/gm	3.74	3.80	3.87	3.94	4.00

[1] Nitrogen Free Extract

Total Collection Technique

Digestion stalls patterned after those of Erwin et al. (1956) were used in the digestion studies. The steers were placed on a high roughage (alfalfa) and low concentrate (barley) diet six weeks prior to the beginning of the experimental period so that the rumen microflora would become adapted to a roughage diet. From time to time during the pre-trial period, the steers were placed in the stalls so that they might become familiar with the surroundings and procedures used in feeding and collecting the feces.

Ration I (100 percent alfalfa hay) was fed for an eight day pre-trial period followed by a six day collection period. Each succeeding ration (II through V) was fed for a seven day pre-trial period and a six day collection period. The rations were fed "as is" without any attempt to standardize protein or TDN intake. Rations were mixed in their proper ratios based on dry matter determinations at the beginning of each digestion trial. The steers were fed at 6:30 A. M. and 4:30 P. M. Feed weights were recorded at each feeding and refused feed was weighed back once a day. A cottonseed meal supplement was fed in equal shares at the morning and evening feeding.

Feces were collected in metal pans placed at the rear of each stall, weighed four times per day and transferred into metal cans.

Composite samples representing about one percent of the total fecal output were taken at the morning and evening feedings and immediately placed in plastic bags for transporting to the laboratory. The samples were frozen and dried at a later date.

Each fecal sample was weighed while still frozen into pre-weighed aluminum pans and dried in an electric oven at 60°C for 72 hours. At the end of this period, samples were reweighed and returned immediately to the oven. If no further loss in weight resulted in 12 hours, samples were considered dry, but if there had been a loss, the samples were dried for an additional 12 hours. After drying, samples were stored in plastic bags until grinding in an intermediate size Wiley mill using a number 20 screen and subsequently stored in bottles until chemically analyzed for ether extract, crude fiber, ash, and crude protein as outlined by the official methods of the Association of Official Agricultural Chemists (1955). Changes were made in the crude protein procedure according to Oldfield (1952) and consisted of collecting the distillate in four percent boric acid and using an indicator consisting of 0.1 percent Bromocresol green in 95 percent alcohol (2 ml). Cellulose was determined by a modification of the procedure of Crampton and Maynard (1938). Tubes containing 0.5 of a gram were boiled in a steam bath for 30 minutes instead of refluxing. The residue was collected in a Gooch crucible containing an asbestos bottom.

Gross energy determinations were completed according to the procedure described by Church (1965) using a Bomb Calorimeter. The routine procedure of using 1 gram samples ground through a 20 mesh screen did not work satisfactorily with fecal samples. To prevent samples from "popping" out when ignited, it was necessary to grind the fecal material through a 40 mesh screen and to reduce the sample size to 0.5 gram. All samples were run in duplicate and those samples that disagreed more than five percent were rerun. All digestion coefficients were calculated on a dry matter basis.

The in vivo cellulose and dry matter digestion coefficients were statistically analyzed using a nested analysis of variance for effects of ration, steer and days (Snedecor, 1956). Standard deviations and coefficients of variation were used to measure the within trial variability of cellulose and dry matter digestion.

Nylon Bag Technique

The nylon bag procedure used in this study is similar to the ones used by Miller (1963) and Ralston, Church, and Oldfield (1962) with modifications existing in the washing and filtering procedure. Samples of the ration were ground in a Abbe 000 mill through a 1/16 inch screen and a ten gram sample was weighed into each nylon bag measuring three and one-half by five inches. Tops of the

bags were secured with a nylon cord drawstring and for extra precaution were tied a second time with a short piece of nylon string. Bags were numbered with blue ink for identification and then attached to loops in a single nylon cord three and one-half feet long tied to an eye bolt in the fistula cap. The cord was suspended into the rumen and allowed to remain free.

Nine bags were placed in the rumen on the morning of the first and fourth collection day of each digestion study. Three bags were removed after 24 hours, three after 48 hours, and the remainder at 72 hours. Three replications and two trials were completed during each six day total collection period.

After removal from the rumen, bags were washed twice in tap water to remove any adhering ingesta, then taken to the laboratory and rewashed in lukewarm tap water. During the washing process, care was taken not to squeeze material from the bag. The bags were constructed of a tight weave containing approximately 120 threads per inch. This allowed only the liquid and minute particles to pass through the walls of the bag. Each bag was washed for exactly one minute, although water coming from the bag was not completely devoid of color when washing was stopped. Each bag was then turned inside-out and rinsed thoroughly using a squeeze bottle. The contents were washed onto a piece of large filter paper. Extreme care was taken to make sure every particle was removed

from each bag. The contents of the bags were allowed to remain on the previously weighed filter paper for approximately 30 minutes until all of the liquid had filtered through. The paper and residue were then dried for 24 hours at 90°C, cooled in a dessicator, and reweighed. Dry matter digestibility was determined by difference between original and digested sample. The residue was used for cellulose analysis and together with dry matter served as a criteria for measuring ration digestibility as affected by length of fermentation period, steer, and ration.

A split plot design as outlined by Cochran and Cox (1950) was used in analyzing cellulose and dry matter digestibility for the following effects:

Factors	Degrees of Freedom	Comparisons
Whole Plot:		
2 Steers	1	Steer A and Steer B
5 Rations	4	Rations I through V
Error	4	
Sub-Plot:		
3 Fermentation Periods	2	24, 48, and 72 hours
Ration X Ferm. Periods	8	Interaction
Sub-Plot Error	40	
Total	59	

This design results in a less precise estimate of the whole plot factors, but a more precise estimate of the sub-plots and interactions. Means, standard deviations and coefficients of variation

were calculated according to Snedecor (1956) and used in measuring the within and between trial variability.

In Vitro or Artificial Rumen Technique

The in vitro apparatus used in this study was patterned after an artificial rumen system developed by Church and Petersen (1960) and modified by Smith (1963). One gram of substrate, ground in a Wiley mill (40 mesh screen) was incubated in each of 60, 250 ml. centrifuge bottles held in two water baths maintained constant at 39°C. These bottles were connected in series of four with looping rubber tubing attached to polyethylene tubing which was inserted through two-holed rubber stoppers. Carbon dioxide was bubbled through the flasks for a period of 15 minutes at the beginning of each trial. The flasks were then disconnected from the series leaving the stoppers intact and allowing the rubber tubing to serve as valves. Moisture accumulation from inside the bottle, combined with the pressure of the CO_2 and other gasses being produced during fermentation, prevented air from coming in and served as a pressure release valve. The pH of the contents was checked during the first trial and found to remain close to the desired pH of seven, consequently it was not checked in later trials.

Thirty ml. of a mineral solution or buffer patterned after

Smith's (1963) was used. The solution contained the following:

Salt	(g/liter)
$NaHCO_3$	4.17
Na_2CO_3	8.30
$Na_2HPO_4 \cdot 12\ H_2O$	1.99
KCl	.48
$CaSO_4 \cdot 2\ H_2O$	.04
$MgSO_4$	.002
Urea	.64

The mineral solution was freshly prepared the evening before each run and placed in a drying oven at 39°C. Immediately before the buffer was used, carbon dioxide was bubbled through the solution until the pH reached 6.8 to 7.0. The buffer solution was added to the rumen fluid to form the 50 ml. volume needed for inoculating each flask.

The rumen inoculum was provided by the two fistulated steers being used. This phase was carried out jointly with the digestion trial and nylon bag study. Rumen fluid was collected prior to the morning feeding or approximately 14 hours from the previous feeding. Rumen contents were squeezed by hand into pre-heated quart thermos bottles and transported immediately to the laboratory. The contents were then filtered through four layers of cheesecloth into tall beakers and after standing for approximately 15 minutes at 39°C, the bottom layer was removed by suction. The inoculum

was then mixed with warm distilled water and buffer solution in the following proportions:

15 ml. rumen fluid/flask

Rumen fluid	600 ml
Distilled water	400 ml
Mineral solution	1200 ml
	2200 ml

Fifty ml. of the mixed inoculum was delivered into each flask by the use of an automatic pipette. The pipette had an Eastern Model 1 stirrer attached to keep the solution thoroughly mixed and carbon dioxide was bubbled through in order to maintain the desired pH.

The experimental design for this phase of the experiment paralleled that used for the nylon bag data. A split plot analysis was used as outlined by Cochran and Cox (1950) in which the steers, inoculum source (rations), and incubation periods of 12 and 24 hours were compared using dry matter and cellulose digestibilities as criteria. Three replications and two trials were completed in this phase of the study. The trials began on day one and day five of each of the five digestion trials.

A second study was carried out jointly with this phase. The five rations (substrates) used in the digestion studies were incubated with each of the five sources of inoculum. The variables consisting of two steers, two fermentation periods, five rations, and five

inoculum sources were analyzed by a 2 x 2 x 5 x 5 factorial design (Snedecor, 1956). Data from this study was collected in an attempt to determine the effect of markedly different inoculum sources on substrate digestion in vitro.

Dry Matter and Cellulose Determinations

The dry matter determinations were completed according to a procedure outlined by Church and Petersen (1960). Fermentation contents in the digestion flasks were filtered by suction through previously weighed fritted glass disc crucibles (50 ml pyrex, 40 μ pore size). The crucibles were dried for 24 hours at 100°C, cooled in a dessicator, and weighed. Residue remaining in the crucibles was considered to be the undigested dry matter of each substrate. Cellulose digestibility was used in addition to dry matter as a criterion in comparing the three techniques. Residue left in the crucibles following dry matter determinations was used for the cellulose determinations.

Comparison of the Three Techniques

Even though each phase of the study was evaluated separately, the basic objective was to compare the three techniques using varying roughage-concentrate ratios. A nested analysis of variance was used to compare the means of dry matter and cellulose

digestibility for each technique at each time period. Kramer's (1956) multiple range test was used in grouping the means with unequal numbers according to their significance. Correlations between the various time periods and methods across rations were used to estimate the closeness of relationship among the techniques. These correlations were used to develop regression equations for *in vivo* digestibilities (Snedecor, 1956).

RESULTS AND DISCUSSIONS

In Vivo Digestion of the Five Rations

The in vivo digestion coefficients for the five rations are presented in Table 2. Means for individual animals, calculated from six observations per steer, are presented in Appendix, Table 1.

Table 2. In Vivo Digestion Coefficients of the Five Rations Used in This Study.

Rations	I	II	III	IV	V
Dry Matter %	54.8	62.0	67.1	75.5	83.8
Crude Protein %	63.5	64.2	61.3	65.2	75.0
Cellulose %	63.5	64.6	55.7	45.1	33.6
Crude Fiber %	46.8	41.5	40.4	41.0	32.6
Energy %	51.4	57.6	60.5	71.4	82.9
Ether Extract %	29.9	45.8	51.1	68.9	77.7
TDN[1]	47.7	56.6	58.9	67.9	71.3

[1] TDN = Total Digestible Nutrients.

Dry matter and energy digestibility increased as the roughage to concentrate ratio decreased from 4:0 to 0:4. The ether extract digestibility increased with each increase in concentrate with values ranging from 29.9 to 77.7 percent. Crude protein was not as radically affected by changes in the roughage to concentrate ratios, varying only ten percent between rations I and V. The crude protein digestibilities tended to vary inversely with the protein content of the rations. There appeared to be no relationship

between crude protein utilization and energy levels. These findings are supported by data reported by Stone and Fontenot (1964). Their data indicated that the apparent digestibility of crude protein and ether extract was not influenced significantly by available energy concentration, but digestibility of dry matter, organic matter, energy and nitrogen free extract (NFE) increased ($P<.01$) with each increase in digestible and metabolizable energy.

Several workers have indicated that the protein or nitrogen content of the ration can affect the digestibility of the other chemical components. Moir and Harris (1962) reported decreases in dry matter and crude fiber digestibility when the nitrogen intake fell below 0.74 percent of feed intake. Ralston, Church, and Oldfield (1962) reported contradictory results to that of Miller (1963) while studying protein digestion using fungal protease in enzyme studies. These differences were attributed to the difference in crude protein content of the hays used in the trials. Also, the availability of the protein may be a contributing factor to its rate of digestion. Miller (1963) suggested that protein digestibility appears to be restricted by small amounts of protein found in some mature hays and by the encrusting fibers that protect it from bacterial breakdown. This could partially explain the higher digestibility of crude protein in the all-concentrate rations in this study, since the concentrate was relatively low in fiber content. In any

event, the protein or nitrogen intake did not fall below that which Moir and Harris have indicated as limiting.

Crude fiber digestibility varied 14 percent between rations I (46.8 percent) and V (32.6 percent), while rations II, III, and IV were uniform in their crude fiber digestibility. Cellulose digestion was highest in ration II (64.6 percent) and lowest in ration V (33.6 percent). The inverse relationship noted between crude fiber ($r = -.91$) and cellulose digestion ($r = -.95$) and the digestion of dry matter is probably best explained simply by the low concentration of fiber and cellulose within these rations. Feedstuffs used in this study were also high in soluble carbohydrates which probably depressed the rate of crude fiber and cellulose digestion. In addition, there is the factor of retention time since higher roughage rations are digested slower and remain for longer periods in the rumen. This results in a more thorough degradation of the fibrous constituents.

Total digestible nutrients (TDN) increased as the roughage to concentrate ratio decreased from 4:0 to 0:4. The TDN values were 47.7, 56.6, 58.9, 67.9, and 71.3, respectively, for rations I through V. Haynes et al. (1955) and Pahnish, Stanley, and Shillingburg (1956) reported similar results by showing a linear increase in TDN as the amount of concentrates in their rations

increased to 66 percent. Dowe, Matsushima and Arthaud (1955) reported results indicating that the TDN increased up to a ratio of 1:4 (roughage-concentrate) and then declined beyond this.

Dry Matter Intake

Dry matter intake for the steers during each of the five digestion studies was not significantly ($P<.01$) different. Dry matter intake increased from ration I (13.6 pounds) through ration IV (17.6 pounds) and decreased with ration V (11.8 pounds). Similar work has recently been published by Montgomery and Baumgardt (1965). They proposed that when the nutritive value is increased by adding concentrates that the dry matter intake decreases and energy intake remains constant. In the present trial, this theory seems plausible if one assumes the increase in intake through ration IV is controlled by rumen distension while the intake of ration V is regulated by other factors, i. e., either satisfying the animal's energy needs or by chemostatic and thermostatic mechanisms. Another possible explanation for the low intake in ration V is simply one of palatability. A constant diet of barley could limit feed intake if other feed sources are not available. There were indications that had the animals been maintained on the all-concentrate diet for a longer period of time that a drastic drop in feed intake would have resulted.

Evaluation of the Three Techniques

In order for a digestibility procedure to be of value, the variability existing within and between trials must be assessed. This portion of the study was designed to assess the magnitude of variabilities associated with the effects of trials, steers, rations, and fermentation periods using dry matter digestion as the criterion. Means, standard deviations and coefficients of variation for dry matter digestibility using the three techniques are presented in Appendix, Tables 2 and 3. The distribution of standard deviations for the effects are summarized in Table 3. Means for the nylon bag, *in vitro*, and *in vivo* include six observations per steer.

Within Trial Variation

Standard deviations for the nylon bag, *in vitro*, and *in vivo* dry matter digestibility ranged from 0.02 to 4.16 percent and the coefficients of variation varied from 0.03 to 8.59 percent.

The *in vitro* within trial variability of dry matter digestibility obtained in the present study is comparable to values reported by other workers for similar *in vitro* procedures. The majority of standard deviations were between 1.00-2.00 percent, with only 30 percent of the standard deviations exceeding 2.00. Bowden and Church (1962) determined the within trial variability in 13 trials

Table 3. The Distribution of Standard Deviations for Trials, Steers, Fermentation Periods, and Ration Means Using the Nylon Bag, In Vitro, and In Vivo Digestibility Techniques.

Standard Deviation Range (%)	Trial Means		Steer Means		Fermentation[a] Period Means			Ration Means				
	I	II	A	B	1	2	3	I	II	III	IV	V
Nylon Bag Technique												
0.00 - 1.00	20	13	20	13	5	5	40	25	8	25	17	8
1.00 - 2.00	50	30	47	40	25	55	55	33	50	59	50	25
2.00 - 3.00	17	10	10	20	25	15	10	0	17	8	25	25
3.00 - 4.00	13	47	23	27	45	25	0	43	25	8	8	42
In Vitro Technique												
0.00 - 1.00	15	20	25	10	20	15		13	14	25	25	14
1.00 - 2.00	50	50	40	60	45	55		13	63	63	50	62
2.00 - 3.00	30	20	25	25	20	30		37	25	14	25	8
3.00 - 4.00	5	10	10	5	15	0		37	0	0	0	0
In Vivo Technique												
0.00 - 1.00			0	20					50			
1.00 - 2.00			100	80				100	50	100	100	100
2.00 - 3.00			0	0				0	0	0	0	0
3.00 - 4.00			0	0				0	0	0	0	0

[a] Fermentation periods for the nylon bag were 24, 48, and 72 hours. Fermentation periods for the in vitro were 12 and 24 hours.

using a standard alfalfa hay. They reported a mean digestibility of dry matter in the alfalfa for all trials of 57.8 percent with a standard deviation of 1.9 percent and coefficients of variation of 3.3 percent using the in vitro technique. Smith (1963) reported standard deviations slightly lower than those obtained in this study. His standard deviations for dry matter digestibility were distributed primarily between .13 and 1.00 percent.

The variability of dry matter digestibility associated with the nylon bag procedure was of a greater magnitude than that for the in vitro and in vivo techniques. In trial II, 47 percent of the standard deviations were in the range of 3.00 - 4.00 percent. Trial I was less variable with the majority (50 percent) of the deviations in the range of 1.00 - 2.00 percent. Other workers have reported estimates of variability for the nylon bag technique as large or larger than those obtained in this study. Hopson, Johnson, and Dehority (1963) reported coefficients of variation as high as 42.40 percent for dry matter digestibility when alfalfa was digested for only six hours. At a 42 hour digestion, the coefficient of variation was 4.63. Yang, Ingalls, and Thomas (1962) reported standard deviations as high as 13.7 percent when alfalfa silage was used as a substrate. However, Miller (1963) obtained lower values with the same two animals and procedure as used in this study. His standard deviations for dry matter digestibility were 0.90, 0.63,

and 0.45 percent for 24, 48, and 72 hour fermentation periods, respectively.

Differences Between Trials

Differences in dry matter digestibility between trials were compared using the Student's t-test (Snedecor, 1956). In general, there was very little difference between trials (Table 4). The variabilities had a similar distribution across both the in vitro and nylon bag data. The only significant ($P<.05$) difference between trials was found for the 24 and 48 hour nylon bag fermentation periods in ration V. The data in the present trial indicate that the repeatability between trials is not a factor when using these techniques and that accurate results could be obtained with one trial if sufficient replications are used. Smith (1963) reported non-significant differences between trials when using both sheep and cattle as donors of rumen liquor. However, Bowden (1961) observed significant differences between trials for both dry matter and cellulose digestibility in vitro and suggested that the difference in digestibility might be due to the digesting power of the inoculum since no effort was made to control the water intake of the steer prior to collection of the rumen liquor.

Table 4. Means for Percent Dry Matter Digestibility for Trials I and II Using the Nylon Bag, In Vitro, and In Vivo Techniques.

	Ration	Trial I	Trial II		Ration	Trial I	Trial II
Nylon Bag	I	50.9[a]	49.1[a]	In Vitro	I	45.5[a]	43.8[a]
	II	59.6[a]	56.4[a]		II	47.4[a]	50.7[a]
24 hours	III	63.5[a]	63.5[a]	12 hours	III	57.2[a]	53.5[a]
	IV	67.7[a]	68.7[a]		IV	57.3[a]	58.9[a]
	V	57.3[a]	50.6[b]		V	81.3[a]	80.5[a]
	I	58.4[a]	59.6[a]		I	46.3[a]	47.8[a]
	II	65.9[a]	67.2[a]		II	59.5[a]	55.0[a]
48 hours	III	76.5[a]	73.3[a]	24 hours	III	65.5[a]	64.4[a]
	IV	84.1[a]	82.5[a]		IV	67.4[a]	71.9[a]
	V	84.6[a]	78.9[b]		V	88.7[a]	88.9[a]
	I	59.4[a]	60.4[a]	In Vivo[c]	I	54.8	
	II	73.1[a]	74.1[a]		II	62.0	
72 hours	III	80.7[a]	78.0[a]		III	67.2	
	IV	89.4[a]	89.9[a]		IV	75.5	
	V	99.3[a]	97.3[a]		V	83.8	

[a a] No significant difference.

[a b] Means significantly different at the 5 percent level.

[c] Only one trial completed for in vivo.

Animal Differences

Differences in dry matter and cellulose digestibility for steers A and B were tested using the Student's t-test (Snedecor, 1956). There were more differences between steers in the nylon bag study than in either of the other two techniques (Tables 5 and 6). The greatest differences were noted in ration III using 24 and 48 hour fermentation periods which were significant at the five and one percent levels, respectively. Ration V was significantly ($P<.05$) different at both the 24 and 48 hour nylon bag fermentation periods. There was also a significant ($P<.05$) difference between steers A and B in ration IV and V at the 12 hour *in vitro* incubation period, but no significant differences for the 24 hour *in vitro* incubation periods. *In vivo* dry matter digestion for steers A and B were not significantly different in any ration.

There were fewer differences between steers A and B when using cellulose digestibility as a criterion for comparison than when using dry matter. Significant ($P<.01$) differences between steers were found using the nylon bag technique at the 48 and 72 hour fermentation periods in ration III. The cellulose digestibility was different ($P<.05$) between steers A and B at the 72 hour period in the nylon bag data in ration II and different *in vivo* for ration IV.

When pooling dry matter and cellulose digestibility across

Table 5. Means for Percent Dry Matter Digestibility for Steers A and B Using the Nylon Bag, In Vitro, and In Vivo Techniques.

	Ration	Steer A	Steer B		Ration	Steer A	Steer B
Nylon Bag	I	50.7[a]	49.9[a]	In Vitro	I	43.9[a]	44.7[a]
	II	56.6[a]	58.9[a]		II	49.2[a]	47.9[a]
24 hours	III	58.3[a]	66.5[b]	12 hours	III	57.2[a]	58.5[a]
	IV	67.8[a]	68.6[a]		IV	64.1[a]	56.9[b]
	V	57.3[a]	51.9[a]		V	83.1[a]	78.5[b]
	I	59.4[a]	58.7[a]		I	46.8[a]	47.2[a]
	II	65.3[a]	67.8[a]		II	56.9[a]	58.1[a]
48 hours	III	68.1[a]	80.0[c]	24 hours	III	64.9[a]	69.1[a]
	IV	80.4[a]	86.2[b]		IV	74.4[a]	70.9[a]
	V	84.6[a]	78.8[b]		V	90.1[a]	87.5[a]
	I	59.4[a]	60.4[a]	In Vivo	I	54.1[a]	55.5[a]
	II	71.9[a]	75.5[a]		II	61.0[a]	62.9[a]
72 hours	III	75.5[a]	83.2[b]		III	66.4[a]	67.9[a]
	IV	88.6[a]	90.7[a]		IV	77.1[a]	73.9[a]
	V	98.3[a]	94.3[a]		V	83.4[a]	84.2[a]

[a a] No significant difference.

[a b] Means significantly different at the 5 percent level.

[a c] Means significantly different at the 1 percent level.

Table 6. Means for Percent Cellulose Digestibility for Steers A and B Using the Nylon Bag, In Vitro, and In Vivo Techniques.

	Ration	Steer A	Steer B		Ration	Steer A	Steer B
Nylon Bag	I	40.1[a]	42.4[a]	In Vitro	I	53.2[a]	53.9[a]
	II	40.5[a]	42.3[a]		II	43.7[a]	48.1[b]
24 hours	III	14.6[a]	19.1[a]	12 hours	III	39.3[a]	36.1[a]
	IV	16.4[a]	15.3[a]		IV	----	----
	V	10.2[a]	9.6[a]		V	----	----
	I	57.3[a]	54.8[a]		I	58.4[a]	57.6[a]
	II	44.1[a]	48.2[a]		II	46.9[a]	49.1[a]
48 hours	III	25.5[a]	42.0[c]	24 hours	III	37.8[a]	38.5[a]
	IV	21.3[a]	30.9[b]		IV	----	----
	V	18.6[a]	20.3[a]		V	----	----
	I	61.5[a]	58.1[a]	In Vivo	I	62.2[a]	64.3[a]
	II	53.2[a]	59.3[b]		II	63.9[a]	65.1[a]
72 hours	III	36.2[a]	50.5[c]		III	55.2[a]	56.4[a]
	IV	35.8[a]	37.9[a]		IV	43.4[a]	46.7[b]
	V	33.7[a]	31.4[a]		V	31.5[a]	35.8[a]

[a a] No significant difference.

[a b] Means significantly different at the 5 percent level.

[a c] Means significantly different at the 1 percent level.

rations (Appendix, Table 4), there were no significant differences between steers. The differences resulting within rations are probably a reflection of only two animals per class. When rations were pooled, a large amount of the individual animal variation within a ration was removed.

The extreme animal differences obtained in ration III is possibly the result of an infection in steer B when he injured his leg on the digestion stall during the third digestion study. He exhibited an abnormal temperature of 104°F. during those days. The steer did not drastically reduce his feed intake so was left in the study for the duration of the collection period. It is possible that the high body temperature had some effect on the nylon bag results, however it was difficult to evaluate this abnormality since neither steer was consistently higher or lower in digestion during the various studies (Appendix, Table 1).

The animal variability was highest for steer B in the nylon bag data (Table 3). Forty-seven percent of steer B's standard deviations were distributed between 2.00 and 4.00 percent as compared to 33 percent for steer A. This could have been one manifestation of the high body temperature. However, the opposite was observed for steer B in the *in vitro* and *in vivo* data. Only five means for each steer were available for *in vivo* comparisons and perhaps the data in Table 3 does not give a valid comparison between

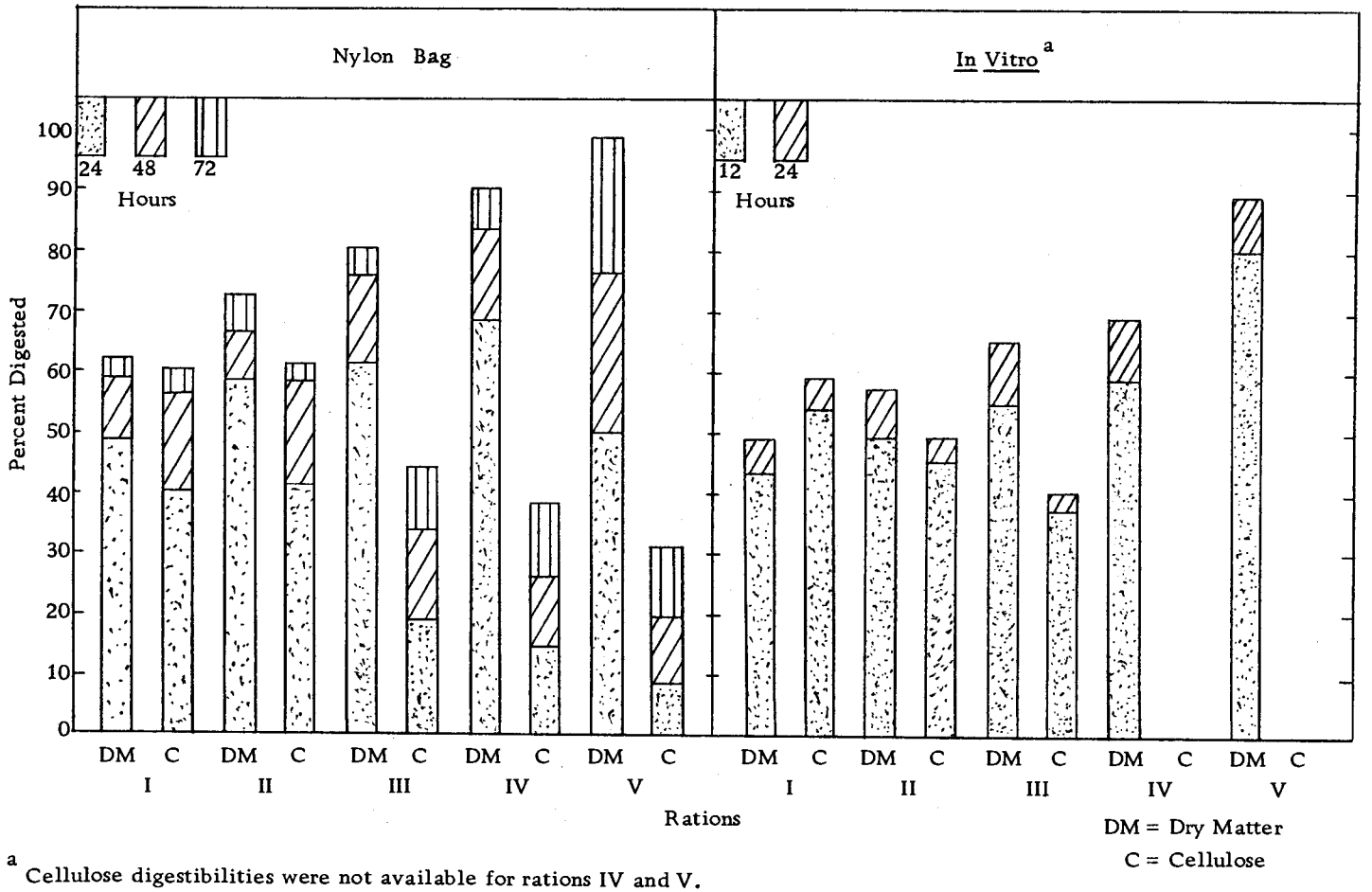
steers, although it is notable that there was generally little variability between animals.

Estimates of variations in digestibility due to animals are limited in the literature. Most in vitro observations include results from a single source of inoculum while nylon bag data generally are reported using only one animal with several replications. However, Van Dyne (1962),using the nylon bag in cellulose studies,reported significant differences when making comparisons between cattle and sheep and when analyzing them within their respective class. He reported differences between steers as great as 12 percent and between lambs of 13 percent, although his results were based on data from only two animals per class. Miller (1963) used the same procedure and steers as in this study and reported no differences between steers A and B. He found means and standard devations for dry matter digestion using a grass hay as a substrate to be $57.4 \pm .60$, $74.2 \pm .51$, and $78.8 \pm .27$ for steer A and 58.1 ± 1.20, $74.1 \pm .75$, and $79.38 \pm .6$ for steer B at 24, 48, and 72 hour digestion periods, respectively. Bezeau (1965),while studying the importance of the ration fed the donor animal for in vitro forage evaluation, noted significant ($P<.01$) differences between two donor animals. He attributed his differences to that of breed and age. Donefer, Lloyd, and Crampton (1961) reported results contrary to these when feeding two donor animals,alternately,

alfalfa and timothy hay and reported no observable difference in cellulolytic response from inoculum prepared from either steer when fed the same forage.

Ration Differences

A comparison of nylon bag and *in vitro* techniques, using dry matter and cellulose digestibility as criteria for the five rations are shown graphically in Figure 1. Nylon bag dry matter digestibility increased through ration IV during both the 24 and 48 hour fermentation periods and then decreased with ration V. The 72 hour dry matter digestion increased through ration V. Cellulose digestion decreased with each increase in percent concentrate. The lower nylon bag dry matter digestion at 24 and 48 hour fermentation periods with ration V is difficult to explain since digestion at 72 hours increased through the all-concentrate ration. There is the possibility that the bags did not mix thoroughly with the rumen ingesta at the shorter fermentation periods since the rumen contained considerable fluid. Had the specific gravity of the bag's contents been greater, the bags might have mixed more thoroughly. It was also notable that the bags contained trapped gas at the shorter periods of digestion, resulting in a "ballooning" effect which caused them to float. However, at the 72 hour fermentation period, the bags appeared to be mixed well with the rumen contents. These data



[a] Cellulose digestibilities were not available for rations IV and V.

Figure 1. Nylon bag and in vitro dry matter and cellulose digestibility for the five rations at the various fermentation periods.

suggests that when digesting high concentrate rations in bags, they possibly should be weighted to the bottom of the rumen to insure maximum exposure to rumen contents and hence uniform digestion. Several workers have suggested this approach (Balch and Johnson, 1950; Miles, 1951; Erwin and Elliston, 1959; and Van Dyne, 1962).

In vitro dry matter digestion increased steadily through ration V. The data for in vitro cellulose digestibility are limited to only the first three rations and follow the same inverse relationship with dry matter, as observed in the nylon bag studies.

Cellulose digestibility for rations IV and V are not reported since an accurate estimation could not be obtained. The higher concentrate rations contained such small quantities of cellulose that it was difficult under the present filtering procedure to separate the fraction of cellulose in the substrate from that in the inoculum. Inoculum dry matter corrections can be made simply by drying a pre-weighed crucible containing the inoculum residue from one flask. This weight is subtracted from each crucible containing the undigested portion of the substrate. Corrections for inoculum cellulose are considerably more involved since 0.5 gram is required for each determination. In order to obtain this sample size, a large quantity of rumen fluid would have to be filtered through a crucible. The amount of inoculum used would then have to be quantified on a flask basis in order to obtain a correction factor.

The variability within rations (Table 3) for nylon bag dry matter digestion was highest in rations I and V. In these rations, approximately 43 percent of the standard deviations were in the range of 3.00 - 4.00 percent. The majority of standard deviations in rations II, III, and IV ranged from 1.00 - 2.00.

The within ration variability in the in vitro data was highest in ration I with 74 percent of the means having standard deviations about 2.00 percent, whereas ration V was the least variable in vitro with only eight percent of the standard deviations above 2.00 percent. The other three rations were more uniform in their distribution of standard deviations, with the majority distributed between 1.00 - 2.00 percent.

The high variabilities for both the in vitro and nylon bag data in ration I may be explained by the nature of the diet. The coarse material in the rumen resulting from the intake of an all roughage diet possibly prevented uniform mixing of the bags and the ingesta. In the in vitro procedure, the variabilities might have been associated with the preparation of the inoculum. It is difficult to say that all flasks received inoculum with the same digesting power. Also, the differences in fineness of grind of the feed sample could have been contributing to the variabilities associated with the in vitro technique. However, the variabilities in both techniques were not of the magnitude to be prohibitive for further use.

Length of Fermentation Periods

Mean digestibility for dry matter and cellulose and percent of total digestion using the nylon bag and in vitro techniques are presented in Tables 7 and 8. Nylon bag dry matter digestion was greatest in the first time period for all of the rations. As the roughage rations were changed to include increments of concentrates, the digestion at 24 hours decreased. In the all-roughage ration, 83.5 percent of the dry matter digestion occurred in the first 24 hours and 15.0 percent in the second period. Only 1.5 percent of the total digestion of dry matter occurred in the last 24 hours. In contrast, 52.5 percent of the digestion occurred in the first 24 hours in the all-concentrate ration, while 27.5 and 19.9 percent dry matter was digested in the 48 and 72 hour periods, respectively. The greater in vitro dry matter digestion also occurred in the first time period in all five rations. Digestion of in vitro dry matter in rations I and V were above 90 percent in the first 12 hours while rations II, III, and IV were digested in an excess of 83 percent in the same time period.

Several workers have studied the effect of length of fermentation period on dry matter digestion using the nylon bag procedure, but, unfortunately, these reports deal only with forages. Van Keuren and Heinemann (1962) observed an increase in percent dry matter

Table 7. Means and Percent of Total Dry Matter Digestion for the Five Rations Using the Nylon Bag Technique at 24, 48, and 72 Hour Fermentation Periods and the In Vitro Technique at 12 and 24 Hour Incubation Periods.

	Dry Matter Digestibility (%)				
	Nylon Bag			In Vitro	
Periods (Hours)	24	48	72	12	24
Ration I					
Mean	50.0	59.6	59.9	44.8	46.9
% Total Digestion	83.5	15.1	1.5	95.3	4.8
Ration II					
Mean	58.8	66.6	73.7	48.9	57.3
% Total Digestion	79.8	10.6	9.6	85.3	14.7
Ration III					
Mean	62.4	74.1	79.4	55.3	64.9
% Total Digestion	78.6	14.7	6.7	85.2	14.8
Ration IV					
Mean	68.2	83.3	89.6	58.1	69.7
% Total Digestion	76.1	16.8	7.1	83.4	16.6
Ration V					
Mean	50.3	76.8	95.9	81.0	88.8
% Total Digestion	52.5	27.5	19.9	91.2	8.8

Table 8. Means and Percent of Total Cellulose Digestion for the Five Rations Using the Nylon Bag Technique at 24, 48, and 72 Hour Fermentation Periods and the In Vitro Technique at 12 and 24 Hour Incubation Periods.

	Cellulose Digestibility (%)				
	Nylon Bag			In Vitro[a]	
Periods (Hours)	24	48	72	12	24
Ration I					
Mean	41.3	56.1	59.8	53.6	57.9
% Total Digestion	69.0	24.7	6.3	92.6	7.4
Ration II					
Mean	41.4	56.2	61.3	45.9	48.0
% Total Digestion	67.5	24.1	8.4	95.6	4.4
Ration III					
Mean	19.3	34.2	43.3	37.7	38.3
% Total Digestion	44.5	34.3	21.2	98.6	1.4
Ration IV					
Mean	15.9	26.1	36.8	----	----
% Total Digestion	43.0	27.8	29.2	----	----
Ration V					
Mean	8.5	19.8	32.2	----	----
% Total Digestion	26.4	35.1	38.5	----	----

[a] Cellulose digestibility for ration IV and V were not available.

digestibility of orchardgrass and sudangrass with each 24 hour increase in length of fermentation period. Alfalfa and ladino clover had much higher dry matter digestibility for the first 24 hour period than the grasses, and increased only slightly through the 48 and 72 hour periods. Yang, Ingalls, and Thomas (1962) reported significantly ($P < .01$) greater dry matter digestibility at 64 hours than at either 44 or 54 hours. Van Dyne (1962) reported that digestion increased with increasing time periods of fermentation up to 60 and 72 hours with the latter being slightly lower than the former. Miller (1963) reported similar findings for dry matter digestibility when using 24, 48, and 72 hour fermentation periods.

Van Soest and Marcus (1964) related the rate of in vivo dry matter digestion in legumes and grasses to the chemical components of the forages. They reported that with legumes one could expect a rapid burst of fermentation initially followed by a plateauing as the soluble cell contents are exhausted. In grasses, a lag period at the start of digestion results because of the smaller amount of highly digestible cell contents. They compared these data to in vitro dry matter digestibility using the same substrates and reported data similar in both magnitude and rate of digestion.

A similar trend to the dry matter data in rate of digestion also was noted for nylon bag cellulose digestion. Cellulose digestion in ration I was 69.0, 24.7, and 6.3 percent for fermentation

periods of 24, 48, and 72 hours, while digestion in ration V was 26.4, 35.1, and 38.5 for the same three periods, respectively. In vitro cellulose digestion in the first 12 hours was 92.6, 95.6, and 98.6 percent for rations I, II, and III, respectively. These data suggest that in vitro digestion periods longer than 12 hours for cellulose would not be necessary when using alfalfa hay as a substrate. Crampton, Donefer, and Lloyd (1959) proposed that a 12 hour in vitro cellulose digestion would most accurately predict the in vivo nutritive value for common legume and grass hays.

Donefer, Crampton, and Lloyd (1959) studied in vitro cellulose digestion at 3, 6, 12, 24, and 48 hour fermentation periods. When compared to leguminous species, grasses displayed lag periods in the start of cellulose digestion. Reid and Jung (1965) substantiated these findings and reported in vitro cellulose digestibilities using alfalfa hay as their substrate. Their data showed that alfalfa fermented rapidly in 12 hours with only a ten percent increase in the next 24 hours. This is comparable to cellulose digestion data reported in the current trial since the greater portion of digestion of the alfalfa substrate occurred in the first 12 hours. Hopson, Johnson, and Dehority (1963) compared nylon bag and in vitro cellulose digestion using alfalfa as their substrate. They reported the rate of digestion for the two techniques to be almost identical when carried out under conditions standardized as to

source of inoculum and length of fermentation period. Van Dyne (1962), using both sheep and cattle, compared the nylon bag technique to in vitro data for various periods of fermentation. He reported almost identical rates of digestion for periods from 24 to 72 hours, using low quality oat hay as his substrate.

The differences in total dry matter and cellulose digestion between the two techniques in this trial may be explained by differences in particle size resulting from substrate preparation. Nylon bag procedures necessitate the need for coarser materials that will not pass readily through the bag walls, while particles for in vitro methods are considerably smaller. Church and Petersen (1960) demonstrated in vitro that the fermentation of alfalfa hay could be altered by using various particle sizes. Their work showed that both dry matter and cellulose digestion ($P<.01$) was reduced as particle size decreased. They suggested that fine grinding could have altered solubilities of other carbohydrate constituents, with a resultant change in cellulose-digesting flora. This might explain the faster rate and lower digestibility observed for the cellulose and dry matter data using the in vitro technique. Erwin and Elliston (1959) digested barley and alfalfa in nylon bags and measured the effect of differences in particle size. They reported an increase in dry matter digestion with fineness of grind, and that the fineness of grind of feedstuff had less effect on digestibility as time of

incubation was increased. However, when Hopson, Johnson, and Dehority (1963) considered this point while comparing the nylon bag and in vitro technique, they reported no differences in either the total digestion or rate of digestion due to differences in particle size. These results lead the author to believe that other factors may then be contributing to the differences in digestion. The weave of the material used in the construction of the bag may have an effect on the digestion since several workers have reported that leaching of material from the bag during digestion and washing can be significant (Yang, Ingalls, and Thomas, 1962; Van Dyne, 1963; and Miller, 1963). Any loss of materials from the bag due to causes other than microbial digestion would be a source of error and would cause higher digestibilities. There is also the possibility that the digesting power of the inoculum decreased sharply after 12 hours. However, this appears unlikely since substantial data are available indicating that this does not occur (Bowden, 1961; Van Dyne, 1962; Smith, 1963; and Hopson, Johnson, and Dehority, 1963).

That the high concentrate rations digested at a slower rate in the nylon bag than in vitro is apparent from data presented for dry matter and cellulose digestion in Tables 7 and 8, respectively. Since substantial information is not available pertaining to the digestion of high concentrate rations using the nylon bag technique, the following discussion is presented as a possible explanation for

these differences. It is apparent that the concentrates, when digested in vitro, followed the established pattern of rapid digestion resulting from the high level of soluble carbohydrates associated with these feedstuffs. It is possible that the bags did not mix uniformly with the rumen contents for reasons expressed earlier (page 46). In rations of this nature, it might be advisable to weight the bags so that they remain in the ventral portion of the rumen to insure uniform digestion. Another explanation for this anomaly is the size of sample used in the bag. Large samples in small bags would tend to "dough up" and not allow the rumen fluid access to the substrate in the center of the bags. Van Dyne (1962) prevented this problem by placing glass marbles in the bags which were intended to keep the material from becoming "doughed" in the corners. A decrease in nylon bag digestion with an increase in sample size has been reported by Van Keuren and Heinemann (1962) and Erwin and Elliston (1959). That the above inherent problems are responsible for the slower rate of dry matter and cellulose digestion with the nylon bag is further substantiated by data presented by the Ohio workers (El-Shazly, Johnson, and Dehority, 1959). They reported biochemical and microscopical data for both in vitro and in vivo (both total collection and nylon bag) techniques. Their work showed a definite parallelism in the rates of fermentation in vivo and in vitro over a 48 hour period as evidenced by a proliferation of

gram-negative micrococci and very small rods in both inoculums. These findings were further substantiated by almost identical rates of volatile fatty acid production for both techniques through 12 hours.

The differences in variability between time periods are presented in Table 3. Forty-five percent of the standard deviations were greater than 3.00 percent for nylon bag dry matter digestion at 24 hours; whereas the standard deviations in the range of 3.00 - 4.00 was 25 and 0 percent for the 48 and 72 hour fermentation periods, respectively. The in vitro dry matter data followed a similar pattern. The 24 hour fermentation period did not deviate above 3.00 percent, while digestion at 12 hours resulted in 15 percent of the standard deviations exceeding values of 3.00 percent. However, in both techniques, the majority of standard deviations were in a range of 0.00 - 2.00 percent.

The above data are substantiated by other workers in regard to the high variabilities associated with the shorter periods of digestion. Hopson, Johnson, and Dehority (1963) reported coefficients of variation of 42.2, 21.4, 14.5, 10.1, 6.8, 5.6, and 4.6 for cellulose digestibility using alfalfa hay when digested for 2, 12, 18, 24, 30, 36, and 42 hours, respectively, using 40 replicates in the nylon bag procedure. Yang, Ingalls, and Thomas (1962) reported standard deviations of six, four, and three percent for alfalfa dry matter digestibility using periods of 44, 54, and 64 hours, respectively.

Standard deviations obtained in this trial using the bag technique, however, did not fall within the limits of data obtained by Miller (1963). His standard deviations, using two steers, were 0.90, 0.63, and 0.45 for the 24, 48, and 72 hour digestion periods, respectively. He suggested that the variability tends to converge at the 72 hour period since it is approaching its maximum digestion, and further postulated that a 48 hour digestion period might be the most accurate since the 24 hour period results in the greatest loss of material from the bag. The leaching of material from the bag during the second and third periods is relatively small and the values obtained would be a result of true "bacterial" digestion.

Working with in vitro cellulose digestibility, Wallace, Rumberg, and Raleigh (1965) reported less within trial variability in the 48 hour fermentation period than in the 24 hour period. Similar results were reported earlier by Bowden and Church (1962) after pilot studies showed less within trial variations in their in vitro procedure when using 48 hour fermentation periods. Barnes et al. (1964),also using in vitro techniques,indicated a reduction in variability with increased length of fermentation periods.

Effects of rations, steers, and trials were pooled and the effect of length of fermentation periods on dry matter and cellulose digestibility was studied within each technique (Appendix, Table 4). Dry matter digestibilities for the nylon bag data were 57.9, 71.9,

and 79.7 for 24, 48, and 72 hour digestion periods, respectively. Digestion at 24 hours was significantly ($P<.01$) different from the 48 and 72 hour periods, but the 48 and 72 hour periods were not significantly different. In vitro dry matter digestibility was different ($P<.01$) for the 12 (57.6 percent) and 24 (65.52 percent) hour periods.

Cellulose digestibility for the pooled data using the nylon bag was 25.1, 38.5, and 46.7 for periods of 24, 48, and 72 hours, respectively. These means were all significantly ($P<.05$) different. In vitro cellulose digestibilities were 45.7 and 48.0 for the 12 and 24 hour periods, respectively, and were not significantly different.

Comparison of the Three Techniques

Differences Between the Means of the Techniques

A comparison of the three techniques using dry matter and cellulose digestion as criteria is presented in Tables 9 and 10. Means were analyzed within each ration using a nested analysis of variance (Snedecor, 1956) and compared using Kramer's (1956) test for grouping means with unequal numbers.

Dry Matter Digestion. Several trends become apparent when studying the range in dry matter digestibilities across these rations of varying roughage to concentrate rations. In vivo digestion of dry matter compares closely to the longer digestion periods in the

Table 9. A Comparison of the Three Techniques Using Dry Matter Digestibility as the Criterion.

Period	Nylon Bag			In Vitro		In Vivo
hours	24	48	72	12	24	
Rations			Percent			
I	50.0^{ab}	59.1^{c}	59.9^{c}	44.8^{a}	47.1^{ab}	54.8^{bc}
II	58.8^{b}	66.6^{cd}	73.7^{d}	48.9^{a}	57.2^{b}	62.0^{bc}
III	62.4^{b}	74.1^{cd}	79.4^{d}	55.3^{a}	64.9^{b}	67.1^{bc}
IV	68.2^{b}	83.3^{cd}	89.6^{d}	58.1^{a}	69.7^{b}	75.3^{bc}
V	50.4^{a}	76.8^{b}	95.9^{d}	81.0^{b}	88.8^{cd}	83.8^{bc}
Pooled	57.9^{a}	71.9^{bc}	79.7^{c}	57.6^{a}	65.5^{ab}	68.0^{b}

a b c d Means on the same line with different superscripts are significantly ($P<.05$) different.

Table 10. A Comparison of the Three Techniques Using Cellulose Digestibility as the Criterion.

Period	Nylon Bag			In Vitro		In Vivo
hours	24	48	72	12	24	
Rations			Percent			
I	41.3^{a}	56.1^{bc}	59.8^{bc}	53.6^{b}	57.9^{bc}	63.5^{c}
II	41.4^{a}	46.2^{a}	56.3^{b}	45.9^{a}	47.9^{a}	64.6^{b}
III	19.3^{a}	34.2^{b}	43.3^{c}	37.7^{bc}	38.2^{bc}	55.8^{d}
IV	15.9^{a}	26.1^{b}	36.9^{c}	-----	-----	48.7^{d}
V	8.5^{a}	19.8^{b}	32.2^{c}	-----	-----	34.0^{c}
Pooled	25.3^{a}	38.5^{b}	46.7^{c}	45.7^{c}	48.0^{c}	53.3^{c}

a b c d Means on the same line with different superscripts are significantly ($P<.05$) different.

higher roughage rations using the nylon bag technique. As the percent concentrate level increases, in vivo digestion compares more closely with the shorter fermentation periods. The in vitro data tended to follow a similar pattern when compared to in vivo data. The 12 hour in vitro digestion period appeared to be of little significance when compared to the in vivo data. Supportive data pertaining to rations of varying proportions of roughages and concentrates as used in this study are not available in the literature. The above pattern of digestion appears plausible, though, when one considers the high level of soluble materials associated with concentrates.

Cellulose Digestion. The in vivo cellulose digestion obtained in ration I was not different from 24 hour in vitro or from the 48 and 72 hour nylon bag digestion. The digestion of cellulose at 24 hours in the nylon bag and 12 hour digestion in vitro underestimated the in vivo digestion. Reid et al. (1960) showed no significant differences between 24 hour in vitro and in vivo cellulose digestion. Similar results were reported by Smith (1963) and Quicke et al. (1959). Lusk, Browning, and Miles (1962) and Van Dyne (1962) found no significant differences between nylon bag digestion at 48 and 72 hours and in vivo digestion of cellulose with both grass and legume hays.

The maximum nylon bag digestion at 72 hours was the only coefficient not significantly different from the in vivo cellulose digestion in ration II. All other digestion coefficients underestimated the in vivo digestion. LeFevre and Kamstra (1960) used a ration consisting of 75 percent alfalfa and 25 percent oats. They reported that 24 hour in vitro cellulose digestion underestimated in vivo cellulose digestion. Similar data were obtained in this study since the 24 hour period was digested significantly ($P<.05$) less than in vivo cellulose.

Cellulose digestion in rations III and IV for both nylon bag and in vitro underestimated the in vivo cellulose digestion. Bowden and Church (1962) reported 48 hour in vitro cellulose digestibility for a 50-50 ration of alfalfa hay and barley of 39.2 ± 3.5. The cellulose digestibility in this trial at 24 hours was 38.2 percent. LeFevre and Kamstra (1960) reported 48 hour in vitro digestion of cellulose to agree closely with in vivo digestion when working with rations containing 75 percent concentrates and 25 percent roughage.

The in vivo cellulose digestion in ration V was not significantly different from the 72 hour nylon bag digestion and 12 and 24 hour in vitro digestion, but was underestimated by the other coefficients.

Pooled Means Across Rations. Pooled means across all rations are presented in Tables 9 and 10 for both dry matter

and cellulose. In vivo dry matter digestion was not significantly different from the 48 hour nylon bag digestion and 24 hour in vitro digestion. These results are similar to those reported for forages by Miller (1963) and Smith (1963). However, these findings are not in agreement with those reported by LeFevre and Kamstra (1960) using a wide range of rations as in this experiment. They reported that over such a range of roughage and concentrates that 48 hour cellulose digestion in vitro would accurately estimate in vivo digestion; whereas, 24 hour incubation periods tended to underestimate the in vivo digestion for both cattle and sheep.

The nylon bag and in vitro cellulose digestibility tended to underestimate the in vivo digestion of cellulose except in ration V. It appears from these data that the nylon bag and in vitro techniques would be of little value in measuring the cellulose digestion in rations containing concentrates if only the means are compared. Similar results using the nylon bag technique in comparisons with in vivo data were reported for cellulose digestibility by Hopson, Johnson, and Dehority (1963).

Church (1961) and Bowden (1961) have proposed that before in vitro or similar techniques can be of value, the procedures must be standardized. Smith (1963) standardized the in vitro procedure used in this study and tested it using various substrates. The same approach must be made concerning the nylon bag technique.

Comparison between methods at various stations are extremely impractical since variations exist in size of bags, weave of material, position of bag in the rumen, size of samples, and many other deviations as discussed in various sections of this thesis. Under the conditions imposed in this research, it appears that the bag and in vitro procedures have definite merits as estimators of digestibilities, although it is apparent from the foregoing data that before the procedures can be routinely used as determinants of digestibility, considerably more data must be collected with a number of feedstuffs.

Relationship Between the Techniques as Measured by Correlations

The previous section compared means by analysis of variance, but did not establish a relationship between the techniques. This section will discuss the relationship between techniques. Comparisons between the techniques and time periods from pooled data are presented in Tables 11, 12, and 13. Correlations for dry matter were determined using ten observations while in vitro cellulose data was limited to six observations since digestion values for rations IV and V were not available. Thus, correlations using the in vitro procedure required greater magnitudes to attain significance. All other comparisons with the various time periods, chemical components, and techniques were completed using ten observations.

Table 11. Correlation Coefficients Between Nylon Bag or In Vitro Dry Matter Digestibility and In Vivo Digestibility of Various Chemical Components.

Factors Correlated	Correlation Coefficient	Regression[a] Equation
Nylon bag dry matter digestibility (48 hours) with:		
In vivo ether extract	.85**	3.2 + 1.52X
In vivo dry matter	.82**	9.1 + 1.00X
In vivo cellulose	-.71*	- 9.7 + .87X
In vivo energy	.85**	- 5.2 + .95X
Nylon bag dry matter digestibility (72 hours) with:		
In vivo crude fiber	-.86**	15.8 + .31X
In vivo ether extract	.98**	-10.6 + .70X
In vivo dry matter	.96**	14.9 + 1.06X
In vivo cellulose	-.85**	-24.4 + .97X
In vivo energy	.93**	0.5 + .80X
In Vitro dry matter digestibility (12 hours) with:		
In vivo crude protein	.78*	44.9 + .31X
In vivo crude fiber	-.87**	22.5 + .30X
In vivo ether extract	.92**	- 4.1 + .83X
In vivo dry matter	.93**	19.5 + .73X
In vivo cellulose	-.96**	- 9.3 + 1.17X
In vivo energy	.96**	14.1 + .77X
In Vitro dry matter digestibility (24 hours) with:		
In vivo crude protein	.70*	45.8 + .26X
In vivo crude fiber	-.94**	19.9 + .31X
In vivo ether extract	.94**	-29.2 + 1.13X
In vivo dry matter	.91**	19.2 + .78X
In vivo cellulose	-.90**	4.6 + .74X
In vivo energy	.97**	17.3 + .80X

[a] Regression equation ($Y = a_o + bX$)

* $P<0.05$

** $P<0.01$

Table 12. Correlation Coefficients Between Nylon Bag or In Vitro Cellulose Digestibility and In Vivo Digestibility of Various Chemical Components.

Factors Correlated	Correlation Coefficient	Regression[a] Equation
Nylon bag cellulose digestibility (24 hours) with:		
In vivo crude fiber	.71*	34.2 + .25X
In vivo ether extract	-.86**	18.9 + 1.06X
In vivo dry matter	-.88**	52.3 + .65X
In vivo cellulose	.86**	24.1 + .72X
In vivo energy	-.83**	47.8 + .68X
Nylon bag cellulose digestibility (48 hours) with:		
In vivo crude fiber	.77**	31.0 + .25X
In vivo ether extract	-.91**	5.0 + 1.10X
In vivo dry matter	-.92**	44.2 + .66X
In vivo cellulose	.81**	38.6 + .67X
In vivo energy	-.80**	38.6 + .71X
Nylon bag cellulose digestibility (72 hours) with:		
In vivo crude fiber	.71*	28.1 + .27X
In vivo ether extract	-.88**	-10.6 + 1.23X
In vivo dry matter	-.88**	35.0 + .74X
In vivo cellulose	.86**	16.6 + .83X
In vivo energy	-.92**	28.9 + .79X
In Vitro cellulose digestibility (12 hours) with:		
In vivo ether extract	-.88**	2.1 + 1.21X
In vivo dry matter	-.94**	26.7 + .75X
In vivo cellulose	.81**	39.7 + .47X
In vivo energy	-.92**	18.9 + .53X
In Vitro cellulose digestibility (24 hours) with:		
In vivo ether extract	-.92**	6.0 + 1.07X
In vivo dry matter	-.97**	30.0 + .64X
In vivo cellulose	.82*	42.4 + .39X
In vivo energy	-.95**	20.9 + .46X

[a] Regression equation ($Y = a_o + bX$)

* $P < 0.05$

** $P < 0.01$

Table 13. Correlations Between In Vitro Dry Matter or Cellulose Digestibility at 12 and 24 Hours and Nylon Bag Dry Matter or Cellulose Digestibility at 24, 48, and 72 Hours.

	12 hours	24 hours
	In Vitro DM Digestibility[a]	
Nylon bag DM Digestibility		
24 hours	.10	.12
48 hours	.71*	.71*
72 hours	.90**	.91**
	In Vitro Cellulose Digestibility	
Nylon bag Cellulose Digestibility		
24 hours	.25	.80*
48 hours	.79	.86*
72 hours	.74	.89*

[a] DM = dry matter
* $P<0.05$
** $P<0.01$

The in vivo digestibility of the chemical components used for these correlations are presented in Appendix, Table 1. Equations (regression equations) were developed from these correlations for the purpose of predicting the in vivo digestibility.

Correlation coefficients obtained between the nylon bag or in vitro dry matter digestibility and in vivo digestibility of the chemical components are shown in Table 11. Nylon bag dry matter digestibility at 48 and 72 hour fermentation periods were highly correlated ($P<.01$) with ether extract, energy, and dry matter digestibility in vivo. The nylon bag dry matter digestibility at 48 hours was correlated ($P<.05$) with cellulose digestibility and highly correlated ($P<.01$) with cellulose and crude fiber digestibility at 72 hours.

The in vitro dry matter digestibility at 12 and 24 hours was correlated ($P<.05$) with in vivo crude protein digestibility and highly correlated ($P<.01$) with digestibilities in vivo of crude fiber, ether extract, dry matter, cellulose, and energy.

Correlations obtained in vitro were in general similar in magnitude to those obtained in the nylon bag procedure. Crude protein digestibility was significantly ($P<.05$) correlated with in vitro dry matter digestibility, but was not related to the nylon bag dry matter data. Bowden (1961) reported statistically significant correlations between the in vitro dry matter digestibility and crude

protein content of the forage. He suggested that the crude protein content may be as good an indicator of the dry matter digestibility of a forage as the in vitro digestibility of dry matter or cellulose.

Correlation coefficients obtained in this study indicated that the nylon bag and in vitro fermentation procedures are definitely related to the in vivo technique and could be used in evaluating the nutritive value of feedstuffs containing varying degrees of roughages and concentrates. Bowden (1961) and Smith (1963) reported similar data for an in vitro procedure while studying forages. Others have reported correlations of similar magnitudes for their in vitro procedures (Asplund et al., 1958; Baumgardt, Cason, and Markley, 1958; and Reid et al., 1959). Similar dry matter data for the nylon bag technique has also been reported (Lusk, Browning, and Miles, 1962, and Van Keuren and Heinemann, 1962).

Correlations in this study at 24 hours were not statistically significant for nylon bag data and are not reported. These data indicate that shorter fermentation periods are too variable to use in comparisons between techniques and are in accord with findings of Hopson, Johnson, and Dehority (1963) who obtained non-significant ($P<.05$) correlations for fermentation periods of less than 36 hours. Their values of 0.52 and 0.54 were significant ($P<.05$) for periods of 36 and 42 hours, respectively. Van Keuren and Heinemann (1962) suggested that periods of 48-96 hours would allow closer correlation

with in vivo digestion data.

The reliability of these correlations might be questioned when considering they were derived from such diverse ratios of roughage to concentrates. Bowden (1961) indicated that his correlations were only of value when differentiating between forages varying widely in digestibility. Other workers have reported similar correlations only when using forages ranging widely in digestibility. Smith (1963) used eight substrates encompassing alfalfa hay to sagebrush, while Reid et al. (1960) used 124 forages from seven stations and Hershberger et al. (1959) used 35 forages of six different species. However, Van Soest and Marcus (1964) improved their correlations between dry matter digestion in vitro and in vivo by grouping their forages into legumes and grasses. Bowden and Church (1962) suggest that a more precise measure of the accuracy of in vitro digestibility is needed when determining the differences in nutritive value within a group of forages. Correlations between techniques were computed within rations in the present study, but the values were generally small and non-significant. Comparisons of in vitro and in vivo data probably have their greatest value for development of equations used to predict the in vivo digestibility of substrates.

Regression equations and correlation coefficients between nylon bag cellulose digestibility at 24, 48, and 72 hours and in vivo digestibility of the rations' chemical components are presented in

Table 12. Highly signficant correlations were obtained between nylon bag cellulose digestibility and in vivo digestibility of crude fiber, ether extract, dry matter, cellulose and energy digestibility at 24, 48, and 72 hours.

Fewer components were significantly correlated with cellulose digestibility using the in vitro technique than the nylon bag (Table 12). Significant correlations were obtained between in vitro cellulose digestibility and ether extract, dry matter, cellulose, and energy digestibility for both the 12 and 24 hour periods.

There is considerable doubt as to the value of the regression equations developed for cellulose from rations containing such wide ranges in roughage to concentrate rations. It would appear more accurate to use dry matter digestibility as a measure of the nutritive value and for predicting in vivo digestibilities since cellulose digestibility is quite low in higher concentrate rations. Small errors associated with the cellulose determinations could have a marked effect on the digestibility estimate.

Correlation coefficients between in vitro dry matter or cellulose digestibility at 12 and 24 hours and nylon bag digestibility at 24, 48, and 72 hour fermentation periods are presented in Table 13. These correlations were computed to evaluate the relationship between these two techniques under standardized conditions imposed in this study. In vitro dry matter digestibility at 12 ($r = .71$) or

24 (r = .71) hours was significantly (P< .05) correlated with nylon bag dry matter digestibility at 48 hours. The 12 (r = .90) or 24 (r = .91) hour in vitro dry matter digestion was highly correlated (P< .01) with the nylon bag dry matter digestibility at 72 hours. In vitro cellulose digestibility at 24 hours was correlated (P< .05) with 24 (r = .80) hour and 48 (r = .86) hour nylon bag cellulose digestibility. The only data similar to that presented above was reported by Hopson, Johnson, and Dehority (1963) when they compared cellulose digestion using dacron bags and the in vitro procedure using alfalfa hay. Although they did not report correlation coefficients, their data showed digestion at the same rate when using periods of 6, 12, 18, 24, 30, 36, and 42 hours. The total amount of cellulose digested in vitro was similar to the total amount digested in the dacron bag. Van Dyne (1962) and Belasco, Gribbins, and Kolterman (1958) have reported similar findings.

The low correlations between the shorter fermentation periods in the dry matter data are probably a result of the higher variabilities associated with these periods. These differences observed at shorter time periods (12 and 24 hours) disappeared in longer fermentations. Johnson et al. (1962) and Donefer, Crampton, and Lloyd (1959) reported similar data when comparing in vitro and in vivo data. The non-significant correlations obtained between the two techniques when using cellulose as a criterion is a result of the

low number of observations (n = 6) used in computing the correlations. There were values as great or greater than those in the dry matter data. These data are indicative that the nylon bag and *in vitro* digestibility techniques are highly related at longer fermentation periods.

The preceding correlation coefficients and regression equations definitely establish a relationship between the three techniques and indicate that each technique would have value in estimating the relative nutritive value of feedstuffs varying in proportions of roughage to concentrates. The question now arises as to which technique would be preferred for digestibility determinations. The procedures in the past have had extremely varied uses. Van Dyne (1962, 1963) has used both the nylon bag and *in vitro* techniques as estimators of range forage evaluation. Ralston, Church, and Oldfield (1962) and Miller (1963) used the nylon bag procedure while studying enzyme reactions in the rumen. Belasco, Gribbins, and Kolterman (1958) used both the nylon bag and *in vitro* procedures to study the response of rumen microorganisms to pasture grasses and prickly pear cactus following foliar application of urea. Reid and Jung (1965) and Hall, Barth, and Hobbs (1958) used the *in vitro* procedure to study the effect of nitrogen fertilizer on digestibility of forages. Bowden (1961) and Asplund *et al.* (1958) studied the production of volatile fatty acid production *in vitro* of forages.

Baumgardt et al. (1964) measured the differences in digestion between steers and goats by using in vitro techniques. Bentley et al. (1954) used the in vitro procedure to study nutritive requirements of rumen microorganisms and Burroughs et al. (1950) used the artificial rumen to study urea utilization by microorganisms from the rumen. The starch digesting ability of rumen protozoa was studied in vitro by Christiansen, Quinn, and Burroughs (1961). Hungate et al. (1960) compared rumen fermentation in European and Zebu cattle using an in vitro procedure while El-Shazly, Dehority, and Johnson (1961) studied the effect of starch on the digestion of cellulose in vitro. Tomlin (1965) used an in vivo bag digestion and lignin content in ranking forages of similar type grown in a single season.

The foregoing literature illustrates the multiple uses of the in vitro and nylon bag techniques. Selection of the technique to use probably depends largely on the information desired, giving consideration to equipment available. The nylon bag technique is useful in field work when relatively small amounts of material are available as little equipment and substrate are required. The in vitro technique is more useful in studies dealing with microbial requirements, volatile fatty acid production, and other studies requiring stricter environmental control. Also, the in vitro procedure enables the digestion of a greater number of feedstuffs since

relatively small amounts of rumen fluid are required per flask. In some instances equipment needed for in vitro procedures may be limiting, although most stations, with some improvising, could provide the necessary equipment. Both procedures are rapid in providing data useful in separating feedstuffs of differing nutritive value and will most certainly find more and more use in the future for determining digestibility of feedstuffs.

Correlations Among In Vivo Digestibilities

Correlation coefficients presented in Table 14 indicate a close relationship between in vivo dry matter digestibility and cellulose digestibility ($r = -.95$), energy digestibility ($r = .98$) and TDN ($r = .98$). In vivo cellulose digestibility was also related to both in vivo energy digestibility ($r = -.97$) and in vivo TDN ($r = -.88$). Also related were in vivo energy and TDN ($r = .96$) digestibility. Smith (1963) reported similar findings and suggested that a reliable method for predicting digestibility of any one of these items should also apply to the other items.

Prediction Equations

A number of workers have successfully used in vitro data as predictors of in vivo digestibility and forage nutritive values. Crampton, Donefer, and Lloyd (1959) and Donefer, Crampton, and

Table 14. Correlations Among Several In Vivo Digestibility Coefficients.

Factors Correlated	Correlations
In Vivo dry matter digestibility with:	
In vivo crude protein digestibility	.74
In vivo cellulose digestibility	-.95*
In vivo energy digestibility	.98**
In vivo TDN digestibility	.98**
In Vivo crude protein digestibility with:	
In vivo cellulose digestibility	-.88*
In vivo energy digestibility	.82
In vivo TDN digestibility	.63
In Vivo cellulose digestibility with:	
In vivo energy	-.97**
In vivo TDN	-.88*
In Vivo energy digestibility with:	
In vivo TDN	.96**

* $P < 0.05$

** $P < 0.01$

Lloyd (1959) were among the leaders in this area. They proposed a method of expressing voluntary intake of a specific forage as a percent of the expected intake of a hypothetical ideal forage. The use of this relative intake value, together with the in vivo percent digestibility, yielded what they termed an "Effective Nutritive Value Index" (ENVI) for numerically describing forages. They suggested that the ENVI (Y) for common legume and grass hays could be predicted from a 12 hour in vitro cellulose digestion coefficient (X) according to the equation $Y = \overline{Y}+b(\overline{X}-X)$ where $b = 1.3$ (X). However, Reid et al. (1960) found that digestibility in vivo was most accurately predicted from dry matter digestibility in vitro of oven-dried samples and that dry matter digestibility in vivo (Y) was related to dry matter digestibility in vitro (X) as expressed by the equation $Y = 20.5 + .778(X)$. They found the correlation between in vivo and in vitro dry matter digestibility to be 0.988. Van Soest and Marcus (1964) developed a prediction equation from correlations between cell contents and the apparent amount digested of feeds ranging from concentrates to highly indigestible wheat straw. They obtained a correlation coefficient of -0.99 and a regression equation of $Y = 15.3 + 0.967X$.

Such prediction equations are derived by the following formula:

$$
\begin{aligned}
Y &= \overline{Y} + b(X-\overline{X}) \\
&= \overline{Y} + bX - b\overline{X} \\
&= \overline{Y} - b\overline{X} + bX \\
&= a_o + bX
\end{aligned}
$$

Where:

A_o is determined from *in vitro* digestibility (X) and is used in the equation to predict the *in vivo* (Y) digestibility of other feeding materials.

Smith (1963) developed equations from *in vitro* dry matter digestibility using eight substrates. His substrates and *in vitro* dry matter digestibilities were used to test the regression equation developed from data in this study. The mean *in vitro* dry matter digestibility, calculated dry matter digestibility, and actual *in vivo* dry matter digestibility are shown in Table 15. The calculated dry matter digestibility was determined by using the equation 9.1 + 1.0(X) developed from the 48 hour nylon bag dry matter digestibility (Table 11). There was not a significant difference between the actual and calculated digestibility using the Student's t-test (Snedecor, 1956).

The same procedure was completed using the regression equation developed from the 24 hour *in vitro* dry matter digestibility (Table 11). The equation obtained in this study was Y = 19.3 + .78X (Table 11). Differences were of about the same magnitude using the equations from the nylon bag and *in vitro* studies and again the

Table 15. *In Vivo* Dry Matter Digestibility as Calculated from Equations Developed Using the Nylon Bag and *In Vitro* Techniques for Dry Matter Digestibility for the Five Rations.

Reference Substrate Smith (1963)	*In vitro* DM Digestibility (X) (1)	Calculated *In vivo* DM Digestibility (Y) (2)	Actual *In vivo* DM Digestibility (3)	Difference (2) - (3)
	%	%	%	%
		Nylon Bag[a]		
Alfalfa Hay	51.6	60.9	59.5	1.4
Alfalfa Hay (Green chop)	50.6	60.0	62.7	-2.7
Meadow Grass Hay 1	56.1	65.5	61.8	3.7
Meadow Grass Hay 2	47.9	57.3	56.6	0.7
Meadow Grass Hay 3	41.9	51.3	51.7	-0.6
Meadow Grass Hay 4	40.4	49.8	49.2	0.6
Mixed Grass Hay	51.7	61.1	62.6	-1.5
Sagebrush	35.1	44.4	53.9	-9.5
		In Vitro[b]		
Alfalfa Hay	51.6	59.5	59.5	0.0
Alfalfa Hay (Green Chop)	50.6	58.8	62.7	-3.9
Meadow Grass Hay 1	56.1	63.1	61.8	1.3
Meadow Grass Hay 2	47.9	56.7	56.6	0.1
Meadow Grass Hay 3	41.9	52.0	51.7	0.3
Meadow Grass Hay 4	40.4	50.9	49.2	1.7
Mixed Grass Hay	51.7	59.7	62.6	-2.9
Sagebrush	35.1	46.7	53.9	-7.3

[a] Equation: $Y = 9.1 + 1.00 (X)$.

[b] Equation: $Y = 19.3 + .78 (X)$.

calculated digestibility was not significantly different (Student's t-test, Snedecor, 1956).

The equations in this study were developed using rations containing high percentages of concentrates and, therefore, their applicability to all-roughage rations is doubtful. However, there is the possibility that they would more accurately predict in vivo digestibilities of rations containing concentrates.

The approach of using equations to predict in vivo digestibility may be questioned by some workers, even though there are considerable data available indicating the reliability of such a procedure. Certainly, more substrates should be digested in vitro to confirm such an approach. It was interesting in this study that the nylon bag was comparable to the in vitro procedure. It appears that both techniques would provide adequate evaluation of feedstuffs of widely varying nutritive value.

The Effect of Inoculum Source on Dry Matter Digestion In Vitro

A number of workers have suggested that rumen inoculum should be obtained from animals receiving a comparable diet to that of the substrate to be digested (Warner, 1956; and Hopson, Johnson, and Dehority, 1963). Gallinger and Kercher (1964), using the nylon bag technique with alfalfa and barley rations, showed that significantly more dry matter and organic matter disappeared from

nylon bags when fistulated steers were fed alfalfa hay than when fed alfalfa hay plus barley. The experimental design of this study presented an excellent opportunity to study the effects of rumen inoculum of markedly different origins on substrates varying widely in composition. Each substrate was incubated in triplicate for 12 and 24 hour periods with each of the five rations serving as inoculum sources. Data pertaining to this portion of the study is presented in Table 16. The data collected at the 24 hour incubation period is discussed in detail since there is less variability associated with longer periods of digestion.

Dry matter digestion of substrate I (100% alfalfa), using the five sources of inoculum, was not significantly different. The digestibility ranged in values from 41.9 percent with inoculum III (50% alfalfa-50% barley) to 47.2 percent with the all-roughage inoculum. The digestion of the other four substrates by the five inoculum sources did not follow the same pattern of digestion as observed with substrate I. There were significant differences between the digesting power of the five inoculum sources. It was of interest to note that the digesting power of inoculum II, III, and IV were very similar across the five substrates, with no significant ($P<.05$) differences between the means.

Digestion of substrates using inoculum I (100% alfalfa) was not greatly different as shown by digestion coefficients of 47.2,

Table 16. Effect of Inoculum Source on Dry Matter Digestibility of the Five Rations.

Inoculum source	I	II	III	IV	V
			12 Hour Dry Matter Digestibility %		
Substrates:					
I	45.3^{a}	40.9^{ab}	36.9^{b}	36.1^{b}	38.9^{b}
II	46.5^{ab}	48.6^{a}	46.1^{ab}	41.1^{b}	49.8^{a}
III	47.4^{a}	55.3^{b}	55.3^{b}	52.1^{b}	63.9^{c}
IV	47.3^{a}	60.9^{b}	59.8^{b}	58.1^{b}	67.4^{c}
V	48.9^{a}	62.3^{b}	65.6^{b}	63.6^{b}	82.3^{c}
			24 Hour Dry Matter Digestibility %		
I	47.2^{a}	44.6^{a}	41.9^{a}	45.5^{a}	45.5^{a}
II	49.5^{a}	57.3^{b}	53.9^{ab}	56.5^{b}	57.9^{b}
III	52.2^{a}	65.3^{b}	65.1^{b}	65.1^{b}	69.8^{b}
IV	53.7^{a}	74.7^{bc}	71.2^{b}	69.7^{b}	79.9^{c}
V	53.5^{a}	79.5^{b}	76.9^{b}	75.7^{b}	88.8^{c}

a b c d Means on the same line with different superscripts are significantly ($P<.05$) different.

49.5, 52.2, 53.7, and 53.5 for substrates I through V, respectively, although the same pattern was not observed for the other four inoculum sources. Digestion of the substrates increased with the addition of each 25 percent increment of concentrate when using inoculums II, III, IV, and V. This is substantiated by a significant ($P<.01$) inoculum substrate interaction (Appendix, Table 5).

In evaluating these data, it is necessary to consider the digestion of a substrate by the same inoculum source as the standard. For example, substrate III digested with inoculum III would be the standard. These standards tended to underestimate in vivo digestion (Table 9) in the first four substrates while digestion in vitro overestimated the in vivo digestion of substrate V.

Data obtained in this study show that all-roughage and all-concentrate rations are digested more completely by inoculums from donor animals on the same diet. Inoculum from animals on a mixed diet (rations II, III, and IV) yielded higher in vitro digestion of substrates of similar composition, regardless of the ratio of roughage to concentrate. A gross relationship between rumen microbes and feedstuffs have not been established in the literature, even though there have been many attempts to classify rumen microflora as to specific action on certain nutrients contained in the feedstuffs. Church (1961) indicated that the rumen microbial population differs considerably between animals maintained on roughage and

concentrate diets. Bezeau (1965) used three sources of alfalfa hay, a grass hay and a native hay and reported that the in vitro digestibility of cellulose was not significantly different where the inoculum was from the same hay. His data further indicated no significant difference between the digesting power of inoculum from the alfalfa hays, but these were superior to both grass and native hays. Specific data to substantiate the above findings are not available; however, in speculation, the author suggests that cellulolytic microorganisms predominate in the all-roughage ration, while starch digesting microbes are in the majority in the all-concentrate ration. The mixed rations would apparently contain sufficient numbers of each to promote adequate digestion of both feedstuffs.

Figure 2 presents graphically the data shown in Table 17. It is interesting to note that substrates I through V varied less than ten percent when digested with an inoculum from an animal maintained on an all-alfalfa diet. As the inoculum source included barley, digestion of the substrates containing barley increased, while the all-roughage substrate declined in digestibility.

The factorial analysis presented in Appendix, Table 5 has been summarized in Table 17. There was a difference in the digesting power of the five inoculums when pooled across the five substrates. Substrates I, II, and III were digested differently by different inoculum sources, but there was no difference between

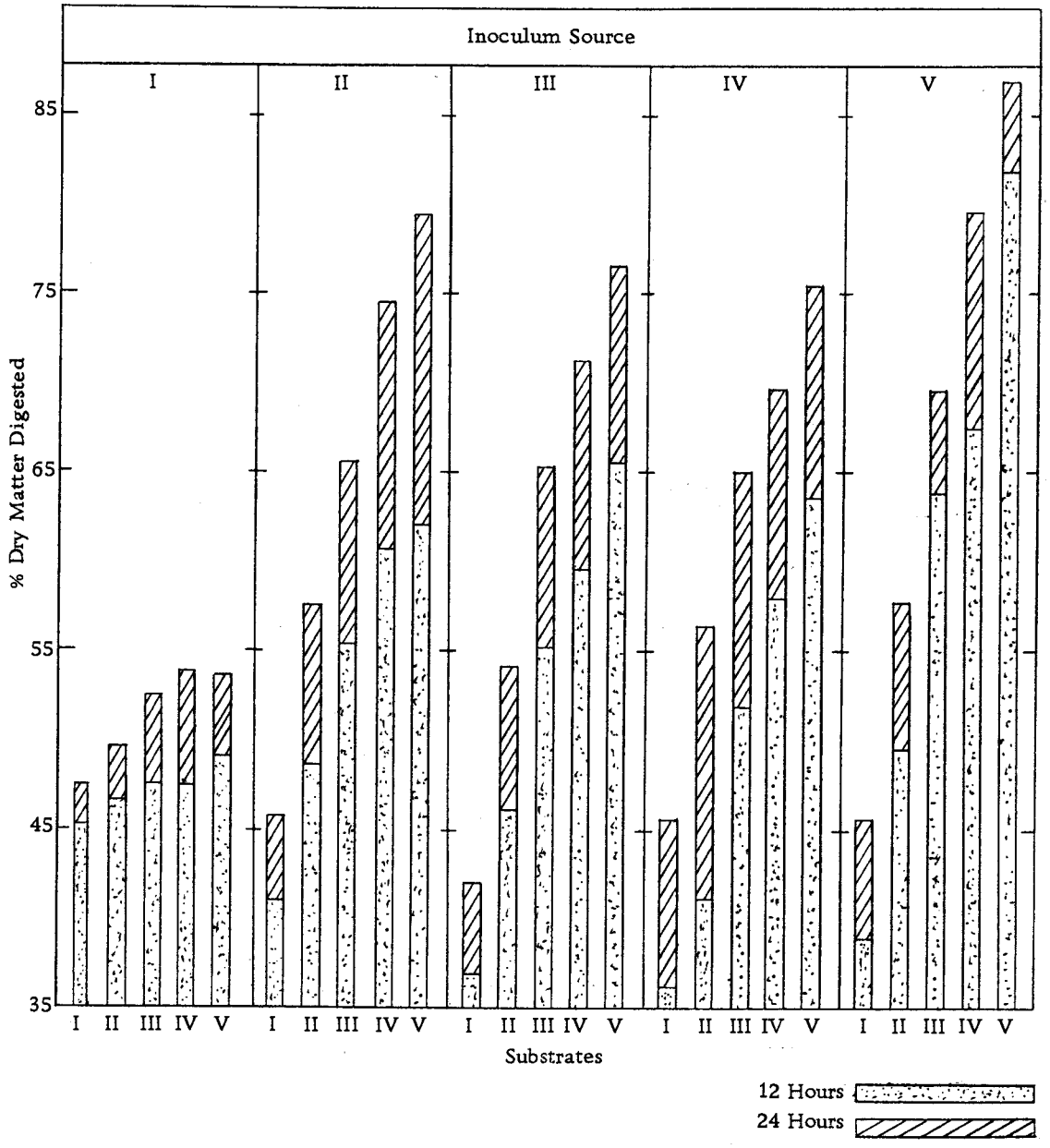


Figure 2. The effect of inocula source on dry matter digestibility of the five rations.

Table 17. Summary of the Effect of Inocula Source on Dry Matter Digestibility of the Five Rations.

Inoculum Source[a]:					
	I	IV	III	II	V
Mean[b] %	49.1	56.7	57.2	59.1	63.5
Substrates:					
	I	II	III	IV	V
Mean %	42.0	50.7	58.9	64.2	69.7
	Steers		Periods		
	A	B	12 hrs.	24 hrs.	
Mean %	58.3	55.9	52.6	61.6	

[a] Means underscored by the same line are not significantly ($P<.05$) different.

[b] Means for inoculum sources I through V are ranked according to their significance.

substrates IV and V. Inoculum sources from steers A and B were not significantly different. Digestion periods of 12 and 24 hours were significantly different ($P<.05$).

The steer X inoculum interaction was significant ($P<.01$) indicating that the steers did not provide inoculum of the same digesting power throughout each of the five inoculums (Appendix, Table 5). Part of this steer effect could have been a result of the infection in steer B while fed ration III. However, when steers were compared there was no significant difference.

The steer X period interaction was not different indicating that increasing or decreasing the length of fermentation periods did not alter the relationship between the two animals. Also the interactions of period X inoculum and period X substrate were not significantly different indicating that the length of incubation period had no effect on the digestion power of the inoculum or rate at which the substrates were digested (Appendix, Table 5). These data are in agreement with that reported by Van Dyne (1962) and Smith (1963).

SUMMARY

The objective of this study was to compare the total collection (in vivo), nylon bag, and in vitro digestibility techniques under standardized conditions. Twin steers were used simultaneously to measure the digestibility of five rations varying in roughage to concentrate ratios. Rations were formulated as follows: 100 percent alfalfa hay (I), 75 percent alfalfa-25 percent barley (II), 50 percent alfalfa-50 percent barley (III), 25 percent alfalfa-75 percent barley (IV), and 100 percent barley (V). One trial was completed in the in vivo study using a six day collection period, whereas two trials were run for each of the other two techniques. Fermentation periods of 24, 48, and 72 hours or 12 and 24 hours were used for the nylon bag and in vitro studies, respectively.

Dry matter, ether extract and energy digestibility and TDN increased as the roughage:concentrate ratio decreased from 4:0 to 0:4. The inverse relationship was noted for cellulose and crude fiber digestibility. Crude protein was not as radically affected by changes in the roughage:concentrate ratios, varying only ten percent between rations I and V.

Dry matter intake increased from ration I through ration IV and declined with ration V. There was no significant difference between feed intake for steer A and steer B.

The within trial variability as measured by standard deviations and coefficients of variation, was highest in the nylon bag technique and lowest in the in vivo technique. The difference between trials, using the nylon bag and in vitro techniques, were generally of little consequence. The greatest difference was obtained in the nylon bag procedure. It was concluded that the repeatability of the trials would not be a factor when using the two techniques if sufficient replications are used.

There were more differences between animals within rations than expected; however, when pooled across all rations, the steers were not significantly different. Animal variations were less when using cellulose digestibility as a criterion than when using dry matter digestibility.

Differences in the digestibility of the five rations were assessed using dry matter and cellulose digestibilities. Nylon bag dry matter digestibility increased through ration IV at both the 24 and 48 hour fermentation periods and then decreased with ration V, while the 72 hour dry matter digestion increased through ration V. Cellulose digestion in the nylon bag decreased with each increase in percent concentrate. In vitro dry matter digestion increased steadily through ration V. The data for in vitro cellulose digestibility was limited to only the first three rations, but followed the same inverse relationship with dry matter digestibility.

The rate and variability of nylon bag and *in vitro* dry matter and cellulose digestion was generally greatest in the first 24 hour period in the five rations and decreased in rate with each increase in time. As the roughage rations included increments of concentrates, the rate of nylon bag dry matter and cellulose digestion decreased in the first period. There was a tendency for cellulose to digest more completely in the first period when using the *in vitro* technique, than when using the nylon bag procedure.

The three techniques, using mean dry matter and cellulose digestion coefficients, were compared by analysis of variance. In general, the *in vivo* dry matter digestion was most favorably compared to the 48 hour nylon bag digestion and 24 hour *in vitro* digestion. The nylon bag and *in vitro* cellulose digestibility tended to underestimate the *in vivo* digestion of cellulose. It appeared from these data that the nylon bag and *in vitro* techniques would be of little value in measuring the cellulose digestion in rations containing concentrates if only the means are compared.

Correlation coefficients were used to establish the relationship between the nylon bag, *in vitro*, and *in vivo* techniques. The nylon bag dry matter digestibility at 48 and 72 hour fermentation periods were significantly correlated with the *in vivo* digestibility of ether extract, energy, dry matter, cellulose, and crude fiber. The *in vitro* dry matter digestibility at 12 and 24 hours was significantly correlated with *in vivo* digestibility of crude protein, crude fiber, ether extract, dry matter, cellulose, and energy. Similar correlations were obtained when nylon bag and *in vitro*

cellulose digestion was correlated with in vivo digestibilities of chemical components. Correlation coefficients between the nylon bag and in vitro techniques indicate a close relationship between the two techniques at the longer periods of digestion. The lower and generally non-significant correlations at the shorter fermentation periods were attributed to the higher variabilities associated with these periods. It was concluded that the three techniques were highly related and could be used in determining the relative value of feedstuffs containing substantial amounts of concentrates.

Regression equations were developed from the preceding correlations. There was no significant ($P<.05$) difference between the predicted and actual in vivo dry matter digestibility of eight substrates when using equations developed from the nylon bag and in vitro dry matter digestibility in this study.

The effect of inoculum source on substrate digestion was studied using the in vitro technique. Data in this study indicated that all-roughage and all-concentrate substrates should be digested with inoculums from animals maintained on the same diet. However, when measuring the digestibility of a mixed substrate (roughage and concentrates), the most accurate results, as measured by a standard, were obtained when the inoculum came from donor animals maintained on a mixed diet regardless of the proportion of roughage and concentrates.

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APPENDIX

Appendix, Table 1. In Vivo Digestion Coefficients of the Five Rations Used in This Study for Steers A and B.

Rations	I	II	III	IV	V
			Steer A		
Dry Matter %	54.1	61.0	66.4	77.1	83.4
Crude Protein %	62.7	64.3	59.8	67.9	75.6
Cellulose %		63.9	55.1	43.4	
Crude Fiber %	47.0	41.7	41.8	39.9	33.8
Energy %	50.8	57.5	60.4	74.1	81.4
Ether Extract %	27.3	43.5	49.9	69.1	78.8
TDN	46.8	58.7	58.6	69.1	71.5
			Steer B		
Dry Matter %	55.5	62.9	67.8	73.9	84.2
Crude Protein %	64.3	64.1	62.9	62.6	74.4
Cellulose %	64.7	65.1	56.3	46.7	35.7
Crude Fiber %	46.5	41.3	39.1	42.0	31.2
Energy %	52.0	57.8	60.6	68.6	84.4
Ether Extract %	32.6	48.0	52.2	68.8	76.7
TDN	48.5	56.5	59.3	66.7	71.0

Appendix, Table 2. Means, Standard Deviations and Coefficients of Variation for Nylon Bag Dry Matter Digestibility at 24, 48, 72 Hours for Trials I and II with Steers A and B.

	Trial I						Trial II					
	Steer A			Steer B			Steer A			Steer B		
Periods	24	48	72	24	48	72	24	48	72	24	48	72
Ration I												
Mean %	51.9	59.5	59.0	49.9	57.0	59.8	46.8	58.7	59.7	48.7	59.7	61.6
S.D. %	3.6	1.2	0.3	3.8	1.1	1.1	3.7	1.3	0.8	3.5	3.1	1.0
C.V. %	7.1	1.6	0.6	7.7	1.9	1.9	7.9	2.3	1.4	7.2	5.2	1.6
Ration II												
Mean %	54.6	66.4	72.8	57.9	67.9	75.2	59.1	64.1	70.4	59.2	67.7	75.7
S.D. %	2.5	1.8	1.6	3.2	1.2	1.1	3.4	1.7	1.1	3.5	2.8	0.2
C.V. %	4.6	2.8	2.3	5.6	1.8	1.5	5.7	2.7	1.6	5.8	4.2	0.2
Ration III												
Mean %	55.1	70.9	76.4	67.6	81.9	84.9	61.4	68.5	74.5	65.4	78.1	81.4
S.D. %	0.8	1.5	0.4	1.9	3.7	1.0	1.7	1.8	1.1	2.1	0.3	1.0
C.V. %	1.4	2.1	0.6	2.9	4.6	1.2	2.8	2.7	1.4	3.3	0.4	1.4
Ration IV												
Mean %	66.3	81.0	89.4	69.1	87.1	89.3	69.3	79.7	87.7	68.0	85.2	92.0
S.D. %	2.7	1.1	0.0	2.0	1.0	0.9	1.9	3.4	1.5	1.3	2.4	1.3
C.V. %	4.1	1.4	0.0	3.0	1.2	1.1	2.8	4.3	1.8	1.9	2.9	1.4
Ration V												
Mean %	57.2	84.6	99.2	53.9	70.0	95.2	57.6	78.9	97.2	49.8	75.6	93.2
S.D. %	2.9	1.6	0.0	1.9	2.0	1.5	3.6	4.1	3.1	3.0	4.1	2.4
C.V. %	5.0	1.9	0.0	4.3	2.8	1.6	7.1	5.2	3.2	6.0	6.7	2.6

Appendix, Table 3. Means, Standard Deviations and Coefficients of Variation for In Vitro Dry Matter Digestibility at 12 and 24 Hours and In Vivo Digestibility for Trials I and II with Steers A and B.

	In Vitro								In Vivo	
	Trial I				Trial II					
	Steer A		Steer B		Steer A		Steer B			
Periods	12	24	12	24	12	24	12	24	Steer A	Steer B
Ration I										
Mean %	43.1	47.2	45.5	45.2	44.7	46.2	42.4	49.0	54.1	55.5
S.D. %	3.7	0.6	2.4	1.4	3.5	2.7	3.2	2.8	1.8	1.6
C.V. %	8.5	1.5	5.3	3.2	7.9	5.8	7.7	5.8	3.4	2.7
Ration II										
Mean %	48.7	57.9	46.1	61.0	50.8	54.9	50.6	55.1	61.0	62.9
S.D. %	2.3	0.8	2.4	1.8	1.6	1.0	1.2	1.3	1.5	0.4
C.V. %	4.8	1.5	5.2	2.9	3.3	1.9	2.4	2.3	2.3	0.7
Ration III										
Mean %	56.4	57.5	57.9	70.7	57.8	69.8	59.0	69.0	66.4	67.8
S.D. %	0.7	1.5	1.8	1.0	0.7	2.4	1.1	1.1	1.2	1.2
C.V. %	1.3	2.6	3.1	1.5	1.3	3.4	2.3	1.9	1.8	1.9
Ration IV										
Mean %	67.1	77.0	57.0	69.0	61.0	71.8	56.7	72.1	77.1	73.4
S.D. %	1.1	1.0	1.0	2.0	2.3	0.3	0.7	1.1	1.3	1.4
C.V. %	1.6	1.3	2.1	3.5	3.8	0.4	1.2	1.5	1.7	2.0
Ration V										
Mean %	86.1	91.2	76.4	86.1	80.5	87.8	80.5	88.9	83.4	84.0
S.D. %	1.0	2.2	1.5	2.3	1.4	1.1	0.4	1.8	1.7	1.9
C.V. %	1.1	2.4	2.0	2.6	1.8	1.2	0.6	2.0	2.1	2.3

Appendix, Table 4. Analysis of Variance for the Split Plot Design in Analyzing the Nylon Bag Data for Dry Matter and Cellulose Digestibility.

Source of Variation	Degrees of Freedom	Mean Square	F Value
Dry Matter Digestibility %			
Whole Plot:			
Steers	1	8.59	0.12 NS
Rations	4	524.02	7.58 *
Error	4	69.14	
Sub Plot:			
Fermentation Periods	2	1211.55	390.82 **
Rations X Fermentation Periods	8	89.40	28.84 **
Sub Plot Error	10	3.10	
Total	29		
Cellulose Digestibility %			
Whole Plot:			
Steers	1	126.64	3.50 NS
Rations	4	1236.61	341.98 **
Error	4	3.61	
Sub Plot:			
Fermentation Periods	2	1000.85	84.03 **
Rations X Fermentation Periods	8	16.68	1.40 NS
Sub Plot Error	10	11.91	
Total	29		

* $P<0.05$
** $P<0.01$
NS Non-significant

Appendix, Table 4 Continued. Analysis of Variance for the Split Plot Design in Analyzing the In Vitro Data for Dry Matter and Cellulose Digestibility.

Source of Variation	Degrees of Freedom	Mean Square	F Value
Dry Matter Digestibility %			
Whole Plot:			
Steers	1	59.51	2.55 NS
Inoculum	4	904.70	387.12 **
Error	4	23.37	
Sub Plot:			
Incubation Periods	1	305.13	116.91 **
Inoculum X Periods	4	15.41	5.90 *
Sub Plot Error	5	2.61	
Total	19		
Cellulose Digestibility %			
Whole Plot:			
Steers	1	1.68	0.31 NS
Inoculum	2	318.84	58.29 **
Error	2	5.47	
Sub Plot:			
Incubation Periods	1	16.29	9.05 NS
Inoculum X Periods	2	3.79	2.11 NS
Sub Plot Error	3	1.80	
Total	11		

* $P < 0.05$
** $P < 0.01$
NS Non-significant

Appendix, Table 4 Continued. Analysis of Variance of Dry Matter and Cellulose Digestibility for the Total Collection Technique.

Source of Variation	Degrees of Freedom	F Value	F Value
		Dry Matter Digestibility	Cellulose Digestibility
Rations	4	45.75 **	41.92 **
Steers/Rations	5	0.06 NS	0.002NS
Days/Rations	10	0.21 NS	5.11 *
Error	10		
Total	29		

* $P < 0.05$
** $P < 0.01$
NS Non-significant

Appendix, Table 5. Factorial Analysis for the Effect of Inoculum Source on the Digestion of the Five Substrates Using the In Vitro Technique.

Source of Variation	Degrees of Freedom	F Value
Total	199	
Observations	1	
Steers	1	1.32 NS
Periods	1	102.57 **
Inocula	4	2.52 *
Substrates	4	17.26 **
Steers X Periods	1	1.87 NS
Steers X Inocula	4	4.46 **
Steers X Substrates	4	.029 NS
Periods X Inocula	4	2.37 NS
Periods X Substrates	4	1.23 NS
Inocula X Substrates	16	5.63 **
S X P X I	4	0.063 NS
S X R X S	16	0.058 NS
S X P X S	4	0.085 NS
P X I X S	16	0.186 NS
S X P X I X S	16	0.124 NS
Error	99	

* $P<0.05$
** $P<0.01$
NS Non-significant