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Techniques of Research In Range Livestock Nutrition

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FOREWORD

Range livestock production is primarily confined to the western region of the United States. The quality and quantity of feed available and consumed by range livestock usually represents the major problem of livestock producers in this area. Several states recognized these problems and initiated studies to elucidate answers on an individual basis but progress was slow because of the complexities of the problems under such widely diverse environmental conditions.

A cooperative approach to these problems was suggested at a preliminary meeting of representatives of most of these states held at the University of Nevada on January 13, 1955. A regional project was developed and initiated on July 1, 1955 titled Range Livestock Nutrition. The objective was "to determine the quantitative and qualitative nutritive value of range forage consumed in terms of chemical analysis, botanical classification, soil, site, stage of maturity, season, drought, and digestibility, relating these factors to reproductive performance, growth and market value of range cattle and sheep." This was revised in 1961 with the following objectives:

(1) To improve techniques for measuring qualitative and quantitative forage intake of range animals and forage digestibility.

(2) To determine the energy, protein, and phosphorus requirements of beef cattle and sheep compatible with various levels of performance on the western range.

The techniques developed or improved by members of the technical committee primarily under the above objectives are included in this regional publication. These techniques are as diverse as the environmental confines of the region in which they were developed but the grouping of available techniques in one publication should greatly aid future research in this area.

The Regional Project W-34 was revised in 1966 and related research is currently continuing as Regional Project W-94 with new research objectives.

Members of the W-34 Regional Committee on Range Livestock Nutrition are:

C. B. Theurer, Arizona
G. P. Lofgreen, California
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TECHNIQUES OF RESEARCH
IN
RANGE LIVESTOCK NUTRITION

Prepared by
L. E. HARRIS, G. P. LOFGREEN, C. J. KERCHER, R. J. RALEIGH
and V. R. BOHMAN, Chairman

GENERAL INTRODUCTION

Most of the land in the western region of the United States is devoted to the production of livestock. These areas produce cattle and sheep that go into the feedlots of the nation and many animals that are sold for immediate slaughter. Much of the national wool production also comes from these states.

Range livestock exist under widely diverse conditions in this region. In the winter they graze semi-deserts or other grasslands or are fed harvested feeds; in the spring and fall they graze foothill pastures or cropland aftermath and in the summer they usually graze on high mountains. These ranges have a diversity of soil, climate, topography, and vegetation. Therefore, the diet of range sheep and cattle is highly variable.

A major part of the animal's feed supply comes from range plants as they are selected in grazing. The diet may be deficient in essential nutrients or may contain an excess of certain constituents that are toxic or poisonous. Drought often occurs which affects both the quality and quantity of forage available. Management practices are also altered under these conditions in an attempt to provide drinking water for grazing animals.

The literature pertaining to range livestock nutrition and techniques for research in range livestock nutrition was reviewed in 1955. The extension of knowledge by a coordinated approach often seems slow but a comparison between knowledge available 12 years ago and knowledge developed primarily by a systematic regional approach since that time emphasizes the progress that has been made. A summary of range livestock nutrition literature available in 1955, when this regional project was initiated, follows:

Harris *et al.* (1952) indicated that research was needed in four phases of range nutrition before adequate recommendations could be made to livestock producers. These include (1) factors that affect the chemical composition of a given range plant specie, (2) the botanical and nutritional composition of the diet of range livestock, (3) supplementary feeding trials on a detailed experimental basis to correct nutrient imbalances in the forage, and (4) supplementary feeding trials on a practical basis using some of the most valuable supplements in various combinations, amounts, and seasons.

The factors which influence the composition of range forage have been reviewed by Cook and Harris (1950a). These authors also conducted a study of a grass, a forb and a browse as affected by vegetation type, site and stage of maturity. Phosphorus and protein decreased and calcium, lignin, and cellulose increased as the plants matured. Soil moisture and site appeared to affect the plant composition more than chemical content of the soil. Similar observations have been made by Archibald *et al.* (1932), Gordon and Sampson (1939), and McCall (1940). Drought may lower phosphorus and protein, whereas calcium and crude fiber may increase (Archibald *et al.*, 1932 and Woodman *et al.*, 1931). Excessive rain after forage matures, leaches out the more valuable nutrients and leaves higher percentages of lignin and cellulose (Guilbert and Mead, 1931; and Hart *et al.*, 1932).

Usually, forage on spring and summer ranges is not deficient in nutrients; however, the fall and winter range forage is often deficient in protein, phosphorus, and energy. In various areas the forage may be deficient in copper, cobalt, and iodine or have an excess of selenium or molybdenum. Under drought conditions and on grass ranges the forage may be deficient in carotene.

Various investigators have found that when the deficiencies in range forage have been corrected, the lamb crop and production of wool have been increased (Marston, 1932; Esplin *et al.*, 1940; Richards, 1942; Meldrum *et al.*, 1948; Whitcomb *et al.*, 1951; Van Horn *et al.*, 1952; and Harris *et al.*, 1956). Black *et al.* (1943) found that the calf crop could be improved by supplementing cattle on a phosphorus deficient range.

Molybdenum occurs in excessive amounts in the forage of

several of the western states, England, New Zealand, and Australia (Russell, 1944 and Maynard, 1951). The level of dietary copper influences the degree of molybdenum toxicity symptoms (Comar *et al.*, 1949). Selenium poisoning has been reported in parts of South Dakota, Montana, Wyoming, Nebraska, and Kansas (Madsen, 1942).

Early attempts to arrive at the grazing animal's diet considered only the major forage plants and the part thought to be consumed by the animal was analyzed chemically. The diet was arrived at by multiplying species composition of the flora by the percentage of each species which has been consumed at the end of the season (Esplin *et al.*, 1937). The obvious necessity for generalization in this method has prevented it from becoming a satisfactory basis for scientific study. An attempt to measure the diet by analysis of the plant species contained in the stomach has also proved unsatisfactory (Norris, 1943).

Cook, *et al.* (1948) adapted the "before and after" method as used by Cassady (1941) and Stapledon *et al.* (1927) to measure the nutrient content of the grazing animal's diet.

Briefly, this method of determining the sheep's diet consists of collecting a given number of specific plant "units" from each species before grazing and a similar number after grazing. These "units" are weighed and chemically analyzed. The difference in weight and chemical composition between the before-grazing and the after-grazing sample serves as a measure of the nutrient content of the ingested forage.

To further evaluate the nutritive intake by grazing animals, it is necessary to determine the digestibility and utilization of the nutrients ingested. The first work of this nature was carried on in Nevada (Kennedy and Dinsmore, 1909). These investigators found that sheep, when fed forage collected from the range, did not show normal selectivity for the plants and frequently did not eat adequate amounts for a maintenance ration. This difficulty was also experienced by Hart *et al.* (1932) and Guilbert and Goss (1944). Because of the difficulties experienced by these investigators, Cook and Harris (1950b) and Cook *et al.* (1951) adapted the lignin ratio techniques of Forbes and Garrigus (1948) and

Ellis *et al.* (1946) to determine the dry matter intake of sheep and the digesibility of the forage.

Early in the digestibility trials it was noticed that forage species such as black sage (*Artemisia nova*) and big sagebrush (*Artemisia tridentata*) had a high content of essential oils resulting in a high ether extract value. This caused the total digestible nutrients and digestible energy to be high. In view of this fact, a urinal was developed for a grazing sheep and the metabolizable energy was determined on several range plants (Cook *et al.*, 1951 and 1954). Reid *et al.* (1950) had developed the "chromogen" technique for measuring digestibility and intake on improved pastures but its applicability to range livestock nutrition had not been ascertained.

Harris *et al.* (1952) devised methods to individually feed sheep and cattle (Harris *et al.*, 1957) on the range.

As studies were designed under this regional project to evaluate range livestock nutrition, it immediately became apparent that adequate techniques and procedures were not available to conduct experiments to provide adequate information. When the project was revised 6 years later, the development of techniques became one of the primary objectives. Techniques have been developed to qualitatively and quantitatively estimate the nutrient intake of grazing range animals. These techniques have been supplemented with *in vitro* measures of forage quality. Techniques have been developed and refined to experimentally supplement animals grazing range forage and to evaluate the nutrient requirements of range animals under a variety of conditions. The purpose of this publication is to review and evaluate the techniques developed and to present the pertinent findings in a complete yet concise form for use by other investigators working in range livestock nutrition and related areas.

ESOPHAGEAL AND RUMEN FISTULA TECHNIQUES FOR QUALITATIVE EVALUATION OF THE GRAZING RUMINANT'S DIET

Summary

Detailed procedures are described for establishing esophageal and ruminal fistulas in experimental animals. Rumen fistulas are easier to establish than esophageal fistulas and animals with rumen fistulas are easier to maintain, although esophageal fistulated animals have been successfully maintained and used for several years. Complete feed collection is probably not possible with the esophageal fistula. The esophageal fistula technique is simple, less time consuming, and is adaptable to both cattle and sheep.

Fistula samples are contaminated with saliva which increases the ash content by adding sodium, potassium and phosphorus compounds to the feed. The nitrogen level of the feed is usually not modified by salivary contamination but may be under some conditions.

Sample preparation of forage samples is critical. The addition of water, artificial saliva or saliva to forage samples followed by drying usually increased the apparent content of lignin and other insoluble carbohydrates, and decreased the soluble carbohydrate portions. These changes were prevented by lyophilizing moistened samples but not by drying under vacuum (25° C) or oven drying (65° C).

Beef cattle and sheep selectively graze range plants. Different plants and different parts of the same plants are preferred. This is reflected in differences in both the chemical and botanical composition of the diet in comparison to the forage available. Frequently the botanical composition of the diet will vary greatly from season to season while the chemical composition will be more constant when animals have the opportunity to selectively graze.

Introduction

Grazing animals have been equipped with either esophageal or rumen fistulas for collection of plant samples for botanical and chemical analyses. Other methods have been attempted which

include various hand sampling techniques, but these methods are subject to human bias. In addition, a skilled technician must be trained in these techniques to obtain useful results. Animal sampling should be more accurate, especially when used by less skilled personnel and thus should be more repeatable. Animal sampling techniques also should be more responsive to changes in quality and quantity of forage available as influenced by intensity of grazing, precipitation patterns, plant growth rates, stage of maturity and especially selective grazing. Animal sampling has only been used to an appreciable extent during the past decade and many problems exist that are inherent with this method. An attempt will be made to delineate these problems and current procedures; to review the literature as it is related to these problems, and to delineate areas where adequate experimental information is lacking.

Esophageal Fistula

A recent review has described the historical development, current operative procedures and use of the esophageal fistula by California workers (Van Dyne and Torell, 1964). Nevada, Idaho (ARS), and Washington workers have used the operative techniques described by Cook *et al.* (1958). Both Washington and California investigators recommend that the most satisfactory location for the esophageal fistula is midway between the head and the body.

The success of the operative procedure and subsequent maintenance of the animal has varied greatly. In some instances 100 percent of the animals have perished within a year and even under good conditions a loss of 10 percent can be expected (Van Dyne and Torell, 1964; Lesperance *et al.*, 1960; Idaho, ARS). Some of the animals have been maintained and used for 2 to 4 years (California and Idaho, ARS). Undoubtedly, the size and type of the fistula cannula are very important in this regard. Animals with esophageal fistulas require 0.5 to 4 hours for sample collection (Van Dyne and Torell, 1964; Lesperance *et al.*, 1960; Price *et al.*, 1964). The length of time usually depends upon the availability of forage. Van Dyne and Torell (1964) and Lesperance *et al.* (1960a) have indicated that one sample per day per animal is usually adequate and multiple daily samples for each animal do not add much additional information. Samples have been collected daily, on alternate days, or monthly, depending mainly on the

experimental demands of the study. The preparation of esophageal-fistulated animals is described as follows:

Pre-operative treatment:

1. Animals are fed high quality chopped forage or grazed on pasture for a few weeks prior to surgery.

2. Feed and water are withheld for a 24 hour period immediately prior to surgery.

Operative procedure:

1. Animals are given a tranquilizer and a local anesthetic (*i.e.* subcutaneous and intramuscular). Care must be taken not to interfere with the swallowing mechanism.

2. Hair or wool is closely clipped from an area extending from the jaw to the brisket on the left side of the neck extending to beyond the ventral midline.

3. The clipped area is washed and disinfected.

4. The animal is placed in a right lateral recumbency with the left front leg drawn back and tied. The rear legs are tied, extended and tied to a post. The head is pulled forward and the jaw elevated by placing a block under the head (see figure 1). The halter rope is tied to a post and an attendant also assists in holding the animal's head in the proper position.

5. A ½ inch steel rod, approximately 3 feet long with a hard rubber ball on the end, is passed down the esophagus to aid in locating the site of the incision and in the blunt dissection through the tissues. Care must be exercised that the rod enters the esophagus rather than the trachea.

6. After the rod is placed in position the block is moved under the neck, lowering the head to allow fluids to drain from the nose and mouth.

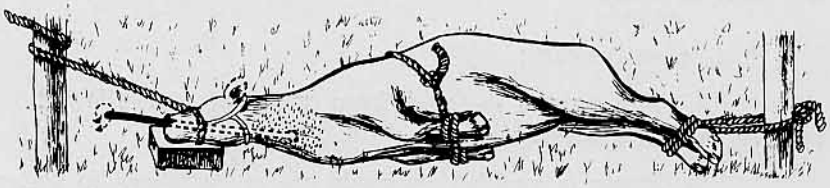


Figure 1. Restriction of animal for surgery with steel rod illustrated in esophagus (courtesy of Dr. G. P. Lofgreen, University of California).

7. The incision is made as near the ventral midline as possible and about midway between the jaw and brisket. The incision site is located with the assistance of the steel rod in the esophagus.

8. An oval shaped piece of skin the size of the fistula is removed and the esophagus exposed by blunt dissection through the covering tissues. The rubber ball on the steel rod provides a base to aid in the dissection. Care is exercised to maintain aseptic conditions as closely as possible and antibiotic powders are placed on the exposed tissue. The size of the fistula in cattle is normally from 1½ to 2 inches long and 1 to 1½ inches wide and the sheep fistula up to 1½ inches long and 1 inch wide.

9. The esophagus is loosened from the surrounding tissues as much as possible and a small incision of about one-half inch is made longitudinally.

10. The edges of the incision in the esophagus are sutured to the submucosa and the inner layers of the skin. The suturing is done with 00 nylon suture with individual stitches ¼ to ⅜ inch apart. The incision is lengthened by ½-inch increments suturing both sides. The procedure is repeated until the entire perimeter of the fistula is sutured. During this process care is exercised to prevent salivary or ingesta contamination of the wound. Regurgitation may be prevented by holding a cotton plug in the esophagus posterior to the fistula. The plug must be held in position to prevent swallowing.

11. The cannula to be used is inserted into the fistula. Some types of cannulas are shown in figure 2.

12. The sutured edges of the fistula are treated with furacin; antibiotics are injected intramuscularly and the neck area sprayed with fly repellent. A surgeon and two assistants can perform the operation on a steer in about one hour and somewhat less time on sheep.

Post-operative care:

1. The animal is freed from restraint and allowed to recover from the anesthetic.

2. Stress is avoided. Animals are given access to water and feed immediately.

3. The same rations fed before the operation are provided. Green grass is best. Care is exercised during the healing process to prevent the consumption of long or coarse roughage which may rip the suture or plug the esophagus.

4. The animals should be inspected frequently the first day or two then daily thereafter. It is not desirable to remove the cannula each day and wash the fistula area.

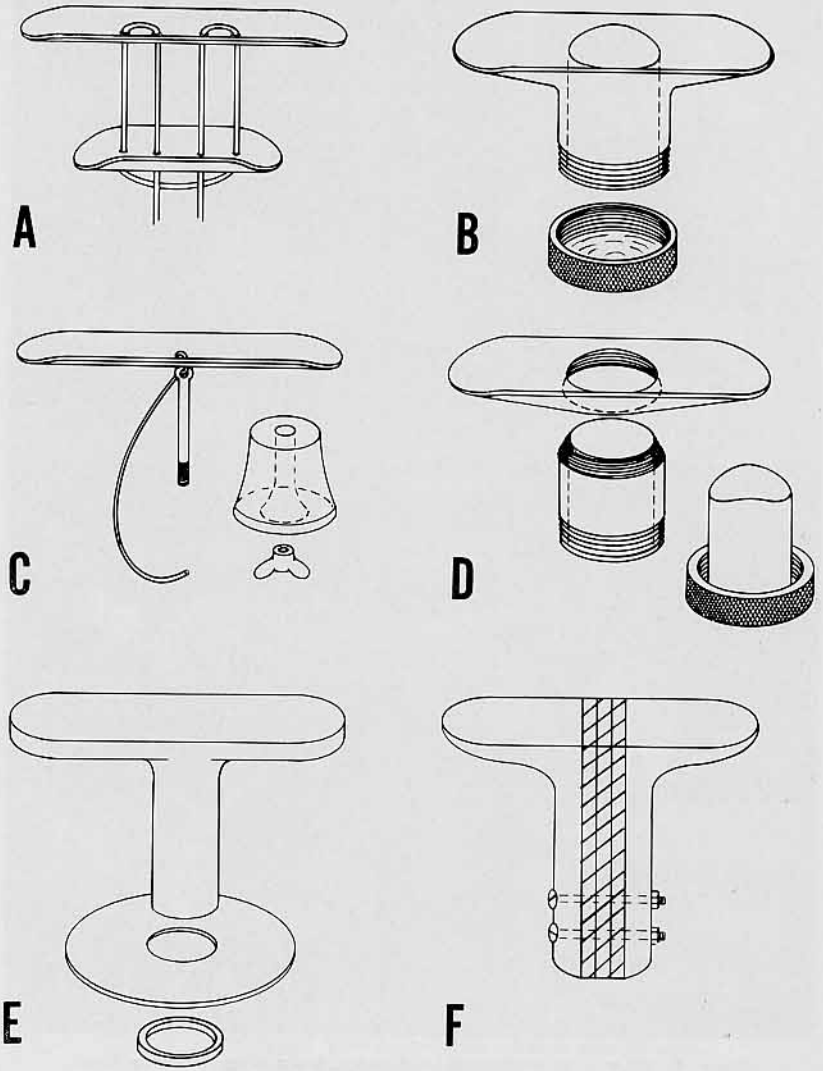


Figure 2. Various types experimental esophageal cannulas (courtesy of G. P. Lofgreen, University of California).

5. About 7 to 10 days after the operation, the sutures are removed. During healing there may be a tendency for the animals to scratch the fistula with the hind leg or on other objects.

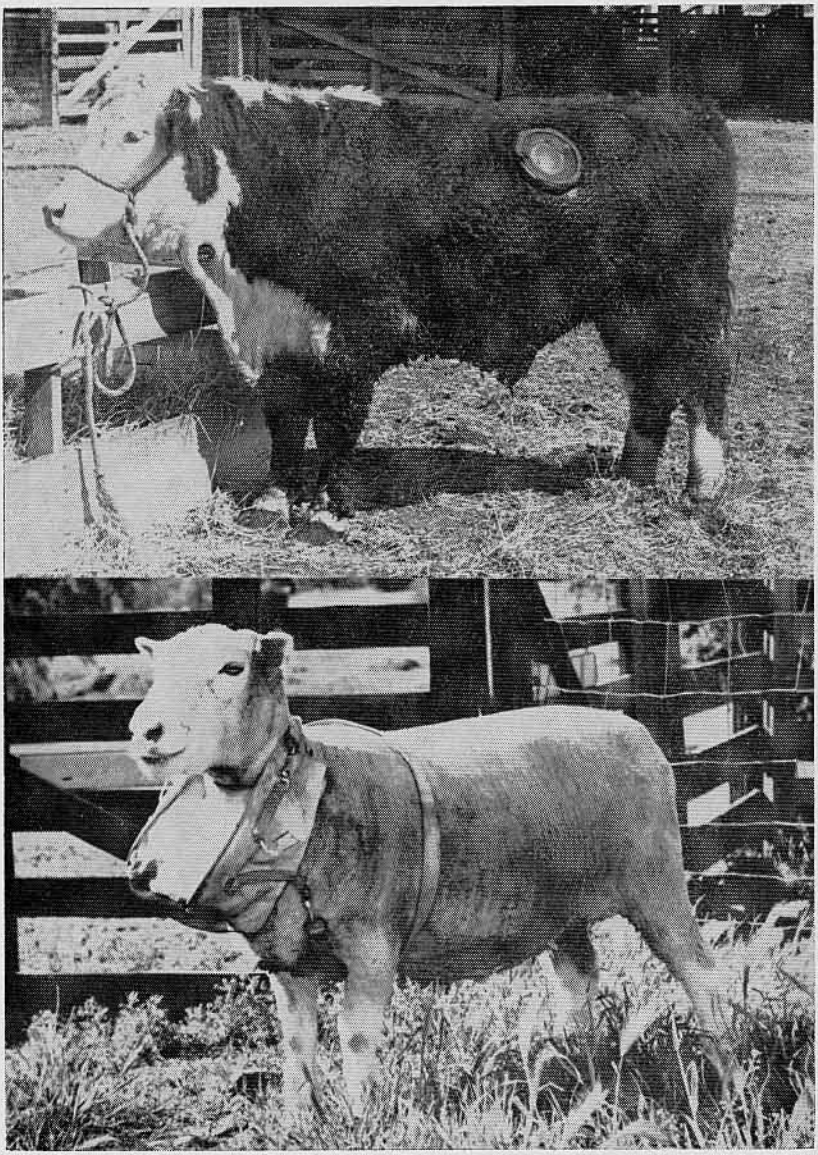


Figure 3. Steer with esophageal and rumen fisula plugs in place (top). Ewe with forage collection bag in place (bottom).

If scratching is a problem, it is desirable to cover the fistula with a piece of canvas approximately 10 inches wide.

6. If the cannula is left in the same position continuously there is a tendency for a pocket to develop anterior to the fistula. Some types of cannulas have a short and long flange on

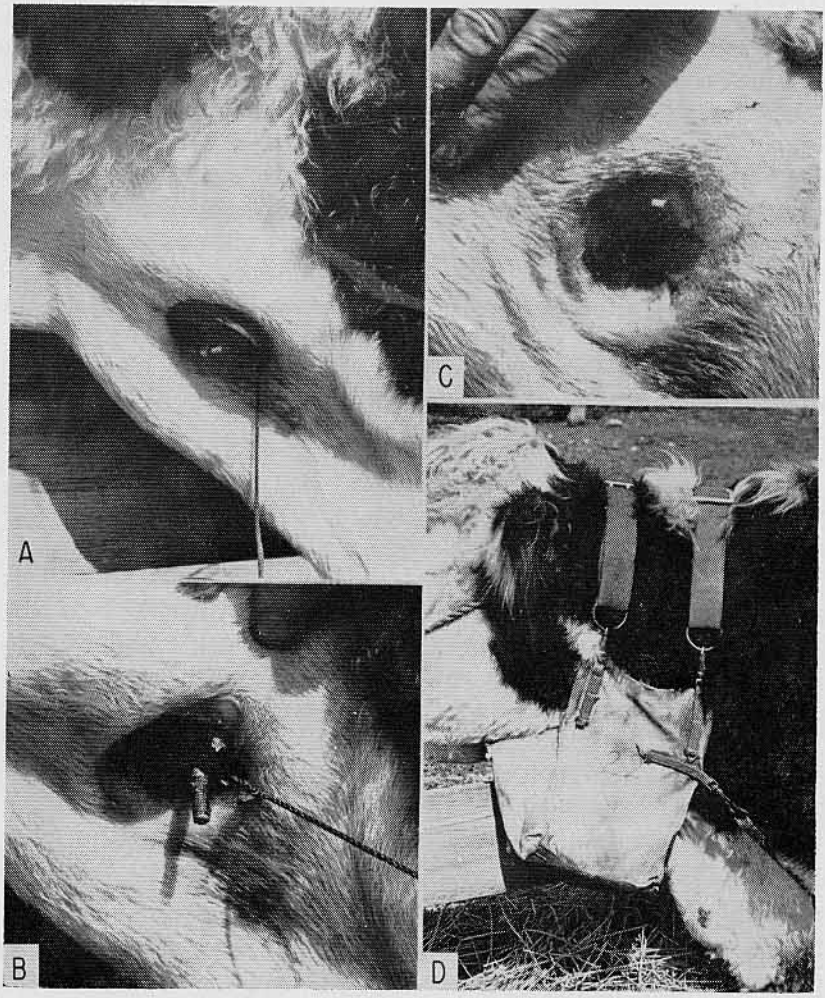


Figure 4. Esophageal fistula with closures and collection bag.
(A) Type-C cannula in place (figure 2).
(B) Type-C cannula in place without external parts.
(C) Open esophageal fistula.
(D) Collection bag in place on fistulated animal.

the interior of the esophagus. With such a cannula the development of a pocket can usually be prevented by reversing the cannula at approximately one to two week intervals. Figures 3 and 4 show the fistulas, cannulas and collection bags on cattle and sheep. For details on types of cannula, collection apparatus and length of sampling time, the reader is referred to Van Dyne and Torell (1964).

Washington workers suggest that fistulated sheep used to collect forage should be hauled to the flock if they are hungry. Otherwise they graze rapidly while approaching the flock. The forage thus collected may not be representative of what other sheep are consuming. Fistulated animals have usually grazed the experimental site for a week or longer prior to sampling to insure representative collections.

Rumen Evacuation Techniques

Rumen fistulas have generally been established by the two-phase method reported by Schalk and Amadon (1928) or a modification of the method of Schnautz (1957).

Many plugs have been developed but the most successful, considering cost, ease of construction, adaptability to rumen evacuation, availability of materials and well being of the animal, is the one employed by Nevada workers. It is constructed by cutting out an eliptical piece of ⅜ inch polyethylene with a ⅜ inch hole in the center. This plate is inserted into the rumen and lined with ½ inch sponge rubber. The rubber lining is larger than the inside plate. An outline of the actual fistula is then formed from a 2-inch board and fitted in the fistula. On the outside, another sponge rubber washer and polyethylene plate is used. These parts are held together by a ⅜ inch bolt with a wing nut on the exterior. Tissue necrosis does not occur with this individually fitted plug, and separate parts may be easily and quickly replaced. Although this plug is not air tight, leakage is slight.

Rumen fistulated cattle have been used extensively at the Squaw Butte Experiment Station, Burns, Oregon. Wooden fistula plugs were not suitable for collection of samples under these conditions because of tissue irritation around the periphery of the fistula. Plastic fistula apparatus, as described by Binns and James (1959), were more suitable. With extensive use the occurrence of

scar tissue around the fistula may necessitate the modification of the rumen cannula. Rumen fistulated steers have been used for forage studies at the Arizona station for 5 years. A vulcanized rubber semi-pneumatic plug has been successfully employed in these studies. This plug has been particularly useful in heavily timbered and brush type ranges. This plug is constructed of butyl rubber. The outside and inside flanges are formed from truck tire inner tubes. The inflatable center core is 4⅝ inches in diameter and constructed of passenger tire inner tube. A valve stem is vulcanized into the center of the end of the outside flange so that the center core can be inflated as needed.

The area around the fistula should be clipped periodically and kept relatively clean from rumen contents that might spill over while evacuating the rumen. Spraying the entire animal and particularly the area around the fistula with a good fly repellent, when needed, is a good precautionary measure.

For sample collection, the contents of the rumen and reticulum are removed thoroughly. Often it is advisable to quickly rinse the rumen with water before sampling. This water may easily be removed by gravity aspiration. A vacuum pump adapted to collect fluid in a 5-gallon drum was used in some Arizona studies to aid in the evacuation of the fluid portion of the rumen contents. Length of sampling period is similar to esophageal fistulas (Lesperance *et al.*, 1960a).

In Oregon, samples were collected by rumen evacuation and by clipping from crested wheatgrass at various dates during two grazing seasons (1960 and 1961) and from native range during two grazing seasons (1961 and 1962). The rumen samples were taken by completely evacuating the rumen and letting the animals graze at will for approximately 45 minutes. Clipped samples were taken concurrently by a technician closely following and observing what the fistulated steers were eating. The rumen contents were well stirred within the rumen and sampled. Both clipped and rumen samples were then dried, ground through a Wiley mill and analyzed for nitrogen and ash. The effect of time interval between collections and the precision of the data collected were not studied. However, the fistulated animals were used every other day on some occasions with no more apparent variation in the data than when they were used weekly.

Table 1. Numbers of animals required for measuring composition of the diet

Constituent measured	Type of forage	Percent mean and confidence level	No. & species of animals+	Type of fistula+	Investigator
Cellulose	Dry, mature Annual range	10% of mean 95% confidence	1 to 2 - SC	E	Van Dyne & Heady (1965b)
Other Carbohydrates	"	"	1 to 6 - SC	E	" "
Crude protein	"	"	1 to 6 - SC	E	" "
Ether extract	"	"	1 to 8 - SC	E	" "
Protein	Tall forb type high mountain summer range	10% of mean 90-99% confidence	1 to 9 - S	E	Price et al. USDA - ARS
Ether extract	"	"	5 to 25 - S	E	"
N. F. E.	"	"	1 to 2 - S	E	"
Lignin	"	"	1 to 6 - S	E	"
Crude fiber	"	"	2 to 6 - S	E	"
Protein	Irrigated pasture	10% of mean 95% confidence level	4 - C	RE	Lesperance et al. (1960)
Botanical composition	Tall fescue	20% of mean 90% confidence	5 - C	R	Ridley et al. (1963)
Botanical composition	Orchard grass	"	11 - C	R	"

+S = Sheep, C = Cattle, E = Esophageal, R = Rumen, RE = Esophageal and Rumen.

The greatest single factor influencing the sample is probably the behavior of the animal. The animals need to be trained for easy handling and the same routine followed while sampling. The same technician should train them and do the sampling.

The numbers of animals required for measuring the composition of the diet under various conditions are given in table 1.

A Comparison of Esophageal and Ruminal Fistula Sampling

Lesperance *et al.* (1960a, 1960b) have conducted the only studies in which direct comparisons of sampling procedures with both esophageal and ruminal fistulas are available. Esophageal fistula samples usually contained more NFE than rumen fistula samples, especially when coarse fibrous roughage was fed. Rumen fistula samples contained more fiber and phosphorus. The composition of both types of samples differed significantly from feed samples and undoubtedly is influenced directly or indirectly from salivary contamination.

Rumen fistulas are easier to establish and maintain than esophageal fistulas (Van Dyne and Torell, 1964; Lesperance *et al.*, 1960a). The sampling procedures in animals with well established fistulas are simpler and less time consuming with esophageal fistulated animals. It would be difficult to make direct comparisons with both cattle and sheep with rumen evacuation techniques, but with esophageal fistula sampling, such comparisons are possible.

The removal of rumen contents for fistula sampling decreased the digestibility of feed from that animal in comparison to digestibility of feed from an intact animal (Connor *et al.*, 1963; Lesperance and Bohman, 1963). Idaho workers obtained similar results. However, this effect was not measurable on improved irrigated pastures (Ridley *et al.*, 1963). This could possibly affect the well-being of the animal and thus influence sampling selectivity. The effect of esophageal fistulation on digestibility by the host animal has not been measured.

Rumen fistula sampling techniques are more adaptable to collection of the complete samples consumed by the grazing animal. It has been suggested that esophageal fistulas do not assure complete collection (Lesperance *et al.*, 1960a). This aspect is not critical if representative sampling is accomplished. Since the nature of eating habits of different ruminants vary, this would be a greater problem with cattle than sheep. Blackstone *et al.* (1965) have only been able to collect 60 to 70 percent of the feed consumed from an esophageal fistula. If feed samples obtained from an esophageal fistula are not representative of the forage consumed at all times, it limits the use of this technique.

The use of fistulated animals for sampling requires that the animals collect a sample while the rumen is empty. The effect of this condition on the selection of plants or plant parts is not known.

Fistula samples can be used to estimate the botanical as well as the chemical composition of the diet. Arizona (Galt, 1966), California (Van Dyne and Heady, 1965), and Nevada (Lesperance *et al.* 1960) investigators have quantitatively identified the botanical composition of cattle and sheep diets with a microscope point method. Galt *et al.* (1966) indicated that single plant species measured from mixtures by rumen evacuation and determined by the microscope point technique were closely related to percent

weight (r^2 equal 0.84 to 0.94) of that species. The microscope point technique was effectively used to estimate the weight of a species mean within 10 percent at the 90 percent confidence level.

Salivary Contamination of Samples

Salivary contamination of forage samples collected by both esophageal and rumen fistulated animals is of great importance in the use of these samples for the nutritional evaluation of range feeds. Van Dyne and Torell (1964) reviewed the available literature at that time and noted that ash was the primary contaminant in fistula samples. Nevada (Lesperance *et al.*, 1960a) and Idaho workers have made similar observations. Phosphorus is the main component of bovine salivary ash of nutritional importance and the level of phosphorus in fistula samples is significantly increased by salivary contamination (Van Dyne and Torell, 1964; Lesperance *et al.*, 1960a). Potassium and sodium were also increased by significant amounts. Calcium is only slightly increased in the collected sample. Grazing animals can also ingest more ash than is present in plant tissues because of contamination of plants with inorganic materials and the mechanical ingestion of soil while grazing. Ash contamination of feed as well as salivary contamination suggests that the composition of fistula samples should be expressed on an ash-free basis. Calculated corrections for salivary contamination are difficult if not impossible under grazing conditions. The amount and composition of salivary contamination varies according to type and amount of feed consumed in addition to other factors. Lesperance *et al.* (1960a) fed various levels of grass hay

Table 2. Composition of samples before and after passing through a sheep esophageal fistula (California)

Kind of sample	Crude protein	P	Ca	Ash
		% of dry matter		
Oats, hay, immature, pelleted				
Before	16.5	0.23	0.35	10.1
After	16.7	0.50	0.36	13.9
Oats, hay, immature, ground				
Before	16.5	0.23	0.35	10.1
After	17.1	0.77	0.42	13.9
Alfalfa, hay, ground				
Before	17.0	0.27	0.98	19.7
After	17.0	1.31	0.94	20.4

to fistulated cattle and measured the amount of saliva produced. For the first pound of hay consumed, 4.5 pounds of saliva were secreted, and for every pound thereafter, up to 3 pounds, an additional 3.6 pounds of saliva were secreted. On succulent forage salivary secretion is not this great.

The nitrogen content of fistula samples was not usually affected in studies at California, Nevada, and Arizona (Bath *et al.*, 1956; Lesperance, 1960a; Shumway *et al.*, 1963).

Idaho (Sharp, 1962), Oregon, and some Arizona studies indicated that the saliva apparently increased the nitrogen content of the fistula sample. Previous treatment and perhaps other factors probably play a role in the nitrogen content of saliva. Oregon studies were based on comparisons of hand-clipped forage with fistula samples and may reflect differences in sampling techniques rather than salivary nitrogen contamination. New Mexico data (Hill, 1965) indicate that rumen samples contained significantly less organic matter but more protein, fiber, and lignin. This may reflect differences both in sampling procedure and preparation.

Although screen bottom collection bags can be used to permit saliva to drain off the esophageal collected sample, there is still residual saliva in the sample. California studies have measured the effect of this contamination on the chemical composition of the sample. Bath, Weir, and Torell (1956) reported a small, but significant increase in ash after chopped alfalfa was passed through the esophageal fistula. The data in table 2 shows that there is a marked increase in phosphorus content of samples passed through a fistula with a small increase in calcium content and a small and perhaps unimportant increase in crude protein. The California data, using samples collected from the fistula, indicate that crude portein, ether extract, lignin, and cellulose were not significantly affected, but that total ash, calcium, and phosphorus were increased 24, 5.5 and 230 percent, respectively, by salivary contamination.

A comparison of feed and fistula samples is shown in table 3 (Lesperance *et al.*, 1960a).

Table 3. A comparison of feed and fistula samples of the same feed[d]

Constituent	Type of feed	Type of fistula	Feed-fistula sample[a]	Regression equation[b]	Correlation value
Crude fiber	Grass hay, alfalfa-conc. pellets	Both	—2.27% ± 0.68**	Y = 2.02 + 0.867X**	.929**
	Alfalfa hay	Esophageal	+4.06% ± 1.77**	Y = 7.94 + 0.900**	.621**
NFE	Grass hay, alfalfa conc. pellets	Both	+4.19% ± 0.91**	Y = 21.04 + 0.590X**	.615**
	Alfalfa hay	Rumen	+1.53% ± 1.36%	Y = 17.99 + 0.614X**	.622**
Energy (kcal/g)	Alfalfa hay	Rumen	+0.059 ± 0.036**	NS[c]	NS[c]
	All types of feed	Esophageal	+0.077 ± 0.028**	Y = 1.509X — 2.132**	.851**
Energy (ash free)	All types of feed	Both	—0.086 ± 0.022*	Y = 2.298 + 0.515X**	.556**
Ash	Grass hay, alfalfa conc. pellets, succulent alfalfa	Both	—2.02% ± 0.38**	Y = 2.22 + 0.628X**	.630**
	Fibrous alfalfa hay	Both	—3.57% ± 0.43	NS[c]	NS[c]
Phosphorus	All types of feed	Rumen	—0.18% ± 0.03**	Y = 0.853 X — 0.110**	.851**
	All types of feed	Esophageal	—0.10% ± 0.02**	Y = 0.867 — 0.054	.970**

* = Significant at $P < .05$.

** = Significant at $P < .01$.

a = Difference is absolute and is determined by subtracting composition of fistula sample from feed sample, i.e., a change of —2.27% indicates that the fistula sample contained 2.27% more of the constituent than did the feed sample. Confidence limits are at the 5% level.

b = X = Fistula sample. Y = Feed sample.

c = NS = Not significant.

d = Lesperance et al. (1960a).

Chemical Changes in Forage Induced by Sample Preparation

Previous work has indicated that the composition of fistula and conventional samples of the same forage differ. This suggests that certain changes occur during the actual fistula sampling or sample preparation. As reported earlier (Lesperance *et al.*, 1960a; Lesperance and Bohman, 1963) these changes include alterations of ash, crude fiber, nitrogen-free extract (NFE) and lignin. Ash contamination can be accounted for completely by salivary contamination (Lesperance *et al.*, 1960). The changes in the carbohydrate fractions are less readily explainable; however, from earlier comparisons of feed and fistula samples of the same feed it is noted that total carbohydrates (crude fiber plus NFE) do not change, although individually, fiber increases and NFE decreases. Apparent dignin also has increased as much as 23 percent during the sampling process (Connor *et al.*, 1963). Excessive drying temperature will increase the apparent lignin percent (McDougall and DeLong, 1942) in forage.

In studies in Nevada (Lesperance and Bohman, 1964), two uniformly mixed hays, alfalfa and grass were sampled four times using the rumen evacuation technique (fistula samples). Artificial saliva or water was added to four other hay samples to approximate salivary contamination of fistula samples. Half of the contaminated samples were oven dried at 65° C and half at 25° C under vacuum (water aspiration). Ash contamination by either

Table 4. Method of moisture removal from contaminated hay samples and its effect upon ash free composition[g]

	Protein	AOAC fiber	NFE	Detergent fiber	Lignin	Total carbohydrates
	%	%	%	%	%	%
Hay	13.6[d]	36.5[d]	47.2[a]	34.3[d]	6.6[a]	83.7[d]
Lyophilized samples	14.2[d]	36.2[d]	47.5[a]	34.0[d]	6.1[a]	83.7[d]
Vacuum dried (25°C)	14.3[d]	39.7[e]	43.5[b]	37.5[e]	6.8[a]	83.2[d]
Oven dried (65°C)	14.7[d]	44.4[f]	39.1[c]	43.7[f]	8.6[b]	83.5[d]

[abc] Means not covered by same superscript differ at $P < .01$.

[def] Means not covered by same superscript differ at $P < .05$.

[g] Lesperance and Bohman (1964).

fistula sampling or artificial saliva was similar, but differed significantly from the other treatments. Artificial saliva, water or fistula samples were of similar composition on an ash-free basis, except fistula samples contained significantly more nitrogen, probably from salivary contamination. Total carbohydrates were not altered by drying methods but fiber increased and NFE decreased. A significant hay type x drying method interaction indicated that oven drying increased the fiber and lignin content of alfalfa more than grass. Vacuum drying lessened these changes more in alfalfa than in grass, but did not eliminate them. Limited observations with lyophilized samples indicated that this method may eliminate those changes in the carbohydrate fraction associated with drying. A sample of this data is shown in table 4.

Arizona studies indicated that drying at 100° C versus 50° C increased the acid-detergent fiber and acid-detergent lignin values of hay. Lowering the pH to 4 before drying did not overcome the adverse effect of the higher drying temperature. Lowering the pH to 4 increased acid-detergent fiber (ADF) and acid-detergent lignin (ADL) values when the hay was dried at 50° C. Treatment of hay with artificial saliva similar to that of McDougall (1948) before analysis lowered the acid detergent values irrespective of drying temperatures.

Selective Grazing

If the botanical or chemical composition of pasture forage differs from forage ingested by the animal, it is an indication of selective grazing. Hardison *et al.* (1954) measured selective grazing by determining the digestibility of hand-harvested and grazed forages. More recently fistulated animals have been employed as biological sampling agents and either the botanical or chemical composition or both of ingested samples compared with the composition of the pasture (Heady and Torell, 1959; Weir and Torell, 1959; Connor *et al.* 1963; Lesperance *et al.* 1960b; Ridley *et al.* 1963b). Lesperance *et al.* (1960b) noted that the botanical and chemical composition of forage harvested from beneath cages and that of the fistula samples collected the same day were not related. The forage selected by animals changed rapidly during a 21-day period while it was relatively constant for cage-protected forage. Ridley *et al.* (1963b) obtained similar results. On orchard grass pastures, containing 40 percent grass, fistulated cattle consistently

selected 35 percent grass; on tall fescue pastures containing 65 percent grass but less palatable, fisulated cattle selected 47 percent grass (table 5) but the amount selected differed from the beginning to the end of the grazing period. Other data on chemical composition of pasture and fistula samples are shown in table 6 (Weir and Torell, 1959). Connor *et al.* (1963) found that cattle selected grass when available on a desert shrub-type range. With

Table 5. Percent orchardgrass or tall fescue in pastures, and fistula samples from cattle grazing the pastures+

Time of pasture sampling	Beginning day	Ending day	Mean
Orchardgrass			
Pasture	40[a]	40[a]	40[a]
Fistula	36[b]	33[b]	35[b]
Tall fescue			
Pasture	66[a]	64[a]	65[a]
Fistula	44[c]	51[b]	47[b]

+ Ridley et al. (1963b).

[abc] Figures with different superscripts within the same category differ significantly at $P < .05$.

Table 6. Chemical composition of forage samples collected by hand clipping and esophageal fistula+

Item	Hopland native range		Hopland improved pasture
	1955	1956	1956
Number of samples			
Hand cripped	9	9	13
Esophageal fistula	19	17	26
	Protein, percent		
Esophageal fistula	14.5	14.8	22.3
Hand cripped	9.5	12.2	18.3
Difference	5.0**	2.6**	4.0**
	Crude fiber, percent		
Esophageal fistula	22.0	21.0	16.2
Hand cripped	27.9	23.5	19.8
Difference	—5.9**	—2.5**	—3.6**

+ Adapted from Weir and Torell (1959).

** Statistically different at $P < .01$.

an average vegetative cover of 90 percent browse, grazing animals consumed from 0.2 to 85 percent grass when measured at monthly intervals. The botanical composition of the diet of animals grazing the same range varies greatly from year to year. This is illustrated in table 7. The amount of rainfall influences both the amounts and kinds of vegetation available. Consequently the diet of grazing animals is modified, but animals appear to have the ability, within limits, to select a nutritively similar diet from the variety of plants available.

Galt (1966) noted that grazing steers were very selective in their choice of available plant species. The botanical composition of the diet, as determined by analyses of rumen contents, varied with time and was not closely related to plant availability or productivity. Protein content of the rumen was considerably higher than the predominant range species, which suggested considerable plant part or species selectivity.

Table 7. Average composition by years of fistula samples at Knoll Creek Field Laboratory, University of Nevada[+]

	Grass	Browse	Forbs	Protein	Annual precipitation[a]
	%	%	%	%	cm
1960	68	24	8	10.9	19.6
1961	82	12	6	11.7	28.2
1962	b	—	—	11.4	25.7
1963	83	0	17	10.7	36.8
1964	93	3	4	10.4	28.4

[+] Bohman and Lesperance (1967).

[a] Calendar year precipitation at Contact, Nevada.

[b] Botanical data not available for 1962.

ESTIMATING DRY MATTER INTAKE AND DIGESTIBLE NUTRIENTS OF THE GRAZING ANIMAL

Summary

Methods for estimating digestibility were investigated using fecal index methods and indicators. The lignin ratio and chromogen techniques were used and compared under varying conditions. Lignin was determined using the 72 percent H_2SO_4 method and the acid-detergent method with varying degrees of success. Apparently saliva contamination, drying temperature, and the pH of the rumen influence the results obtained by the lignin ratio technique. The chromogen method gave varying results with some workers reporting it satisfactory and others finding a wide degree of variation in results. Apparently, if either the lignin ratio or chromogen technique is to be applicable to range conditions, both techniques require further refinement.

Chrominum oxide was used as an external indicator for determining the digestibility and feces output. The most consistent results were obtained when Cr_2O_3 was dispersed on cellulose before administration. When "free" Cr_2O_3 was administered there was an extremely wide range of Cr_2O_3 recovered in the feces.

Feces output of grazing animals was determined using C_2O_3 dispersed on cellulose and administered twice daily with grab fecal samples taken at the same time. Some animals received range forage only and others received supplements several times during the year. Variation among animals was small and intake measurements appeared reasonable.

An *in vitro* technique for determining fecal output and dry matter intake was developed. This procedure involves (1) determining the digestibility of range forage and a standard forage *in vitro* or by nylon bag, (2) predicting the digestion of range forage by a regression equation, and (3) determining forage intake by using the predicted digestibility, the composition of representative range forage and fecal output. Equipment was developed to collect urine and feces of grazing range cattle. Also, the use of fistulated animals for digestibility trials was evaluated.

Estimating Digestibility

Fecal-index methods, lignin ratio, chromogen

The fecal-index technique has been investigated and used by several contributors in connection with this project. The N:dry matter ratio as proposed by Lancaster (1949) and conventional trials have been used for determining digestibility. The chromogen method as proposed by Reid *et al.* (1950) was investigated in Oregon by using hand-fed steers. Its application under range conditions appeared dubious because of the large variation (table 8). Other workers have drawn similar conclusions relative to the application of chromogen pigments in range nutrition (Cook and Harris, 1951, Kennedy *et al.*, 1959 and Wheeler, 1962). However, Nevada workers (Connor *et al.*, 1963 and Ridley *et al.*, 1963) concluded that the chromogen technique was more reliable than the lignin method for determining apparent digestibility and forage intake of range cattle during 3 summer months (table 9). Lignin by the Thacker method (1954) had an apparent digestibility of 47.5 percent and lignin in fistula samples averaged approximately twice that in hand harvested samples. No suitable equation for correcting these values could be derived.

Workers at the U. S. Sheep Experiment Station at Dubois, Idaho estimated the intake and digestibility of sheep forage on

Table 8. Average percent recovery of chromogen pigments from hand-fed steers.

Trial No	Steer No	Recovery %
1	2	70.5
	4	72.2
	6	72.0
	Mean	71.6
2	2	68.7
	4	67.5
	6	67.8
	Mean	67.7
3	2	96.3
	4	87.0
	6	108.9
	Mean	97.4

Table 9. Apparent digestion coefficients for dry matter as determined with three indicators

Period	Season+		
	June	July	August
Northern Nevada			
Lignin	43.6[a]	25.6[a]	8.2[a]
Corrected lignin §	60.8[b]	62.1[b]	47.1[b]
Chromogens	71.4[c]	67.2[c]	58.4[c]
Southern Nevada			
Lignin	11.3[a]	15.8[a]	8.5[a]
Corrected lignin	38.9[b]	42.7[b]	39.7[b]
Chromogens	54.5[c]	57.9[c]	28.1[c]

+ Values with different superscripts, (a, b, c), if within the same period are significantly different ($P < .01$) except that chromogens differ ($P < .05$) from the lignin indicators during mid-season at southern Nevada. Each value represents the average of six animals (five during period 1) for northern Nevada.

§ Calculated from a regression equation developed from 78 samples of forage before and after fistula sampling.

high-mountain summer range by using the lignin ratio technique and total fecal collection. Lignin was determined by the 72 percent H_2SO_4 method and by the acid-detergent lignin (ADL) method (Van Soest, 1964). Digestibility values for crude protein, ether extract, crude fiber, nitrogen free extract, dry matter, and gross energy were consistently higher when the ADL method was used. Estimates of dry matter intake were consequently also higher.

Arizona studies suggest that ADL (Van Soest, 1964) can be used as an internal indicator for estimating forage nutrient digestibility if minor corrections are made for the limited amount of lignin digested. Preliminary studies show that 4 to 8 percent of the lignin in alfalfa hay is digested by steers. In these studies, saliva contamination decreased ADL while lowering the pH to 4 or increasing drying temperature from 50 to 100° C caused an increase in acid-detergent lignin.

External indicators

Wyoming, New Mexico, Nevada, and Oregon have worked with chromium oxide (Cr_2O_3) for determining fecal output. In the Wyoming work, yearling steers grazing native shortgrass range were given 5 gram capsules of Cr_2O_3 at 6 a.m. and 6 p.m. Three trials were conducted during September, with cured forage, in 3 successive years and one trial was conducted in June on green grass.

Fecal collection bags were used for total collection and "grab samples" were taken at 3-hour intervals from 6 a.m. to 6 p.m. The concentration of nitrogen, chromic oxide, and chromogens in the "grab samples" and in the total collection samples did not follow any definite pattern related to time of collection. The chromic oxide recovery in the feces was variable and ranged from 61.9 to 105.4 percent. As a result, predicted fecal dry matter excretion was higher than actually occurred.

To estimate fecal dry matter excretion, New Mexico workers administered chromic oxide-impregnated paper daily and every other day to steers fed prairie hay and cottonseed meal. In the first trial, 15.35 grams of chromic oxide were administered once daily, (6 a.m.) by using a balling gun. Uniform fecal excretion of chromic oxide began on the third day after initial administration. The recovery of chromic oxide for days 3 through 11 was 102.5 percent (table 10). Neither differences among steers nor among days were significant. During the next 48 hours the diurnal excretion pattern was studied by determining the chromic oxide concentration of feces grab samples obtained from the rectum of

Table 10. Recovery of chromic oxide impregnated on paper administered daily and every other day to yearling steers

Day	Cr_2O_3 recovery with daily administration	Day	Cr_2O_3 recovery with every other day administration
	%		%
1[a]	22.1	1[b]	30.4
2	65.4	2	85.0
3	112.1	3	85.9
4	109.6	4	99.8
5	104.1	5	76.8
6	106.8	6	95.5
7	101.1	7	69.4
8	91.6	8	110.1
9	99.4	9	72.8
10	96.9		
11	102.4		
Mean for days 3- 11	102.5	Mean for days 2, 4, 6, 8[c]	97.6
		Mean for days 3, 5, 7, 9[d]	76.2

[a] 15.35 g Cr_2O_3 daily.

[b] 30.70 g Cr_2O_3 every other day starting with day 1.

[c] 4 days of Cr_2O_3 administration.

[d] 4 days following administration.

each steer at 2 hour intervals. The concentration of chromic oxide in grab samples collected 3 to 18 hours after administration represented approximately 100 percent recovery. During the next 5 days, grab samples were collected 30 minutes after chromic oxide administration. Diurnal variation data indicated that chromic oxide concentration of the fecal samples collected at this time should be corrected to 100 percent recovery by using the factor 1.126. The estimated fecal output and dry matter intake using lignin as an indicator is in close agreement with the measured values.

In a second trial, 30.70 grams of chromic oxide were administered every other day. On the second day after administration, the recovery of chromic oxide was 85.0 percent which was uniform and near the mean of the following 7 days. The recoveries were 97.6 percent for the 4 days of administration and 76.2 percent for the 4 days after administration. These recoveries were significantly different ($P < .05$) but the difference among steers and among days was not significant.

In a 4-day study of diurnal variation the fecal chromic oxide concentration declined during the 24-hour period immediately following administration and rose during the second 24-hour period. The sampling times when chromic oxide concentration was equivalent to 100 percent recovery were about 2 and 30 hours after administration. Diurnal variation data indicated that the chromic oxide concentration for the grab samples taken on the day of administration should be adjusted by the factor 0.971.

When diurnal variation was studied for 6 days with only two grab samples per steer in a 24-hour period, the pattern of fecal chromic oxide concentration was similar to that when each steer was sampled every 2 hours.

Nevada researchers (Lesperance and Bohman, 1963) reported the prediction of total fecal output by using Cr_2O_3 and grab samples compared favorably with that determined by total fecal collections. They found that the Cr_2O_3 grab sample technique overestimated fecal output by 0.4 percent resulting in a correlation of $r = .920$ with total collection determinations. However, these results were obtainable only when the determination of Cr_2O_3 was based on standards prepared in the presence of fecal ash. Determinations based on Cr_2O_3 standards without fecal ash overesti-

mated fecal excretion by 14 percent. Cr_2O_3 was administered twice daily and fecal grab samples were taken at the same time.

A major portion of the Oregon work on this project was directed toward use of Cr_2O_3. In the early stages of this work Cr_2O_3 was administered once daily to steers grazing on hand-fed *Agropyron desertorum* in three separate trials. The percentage of fecal recovery of the indicator for these trials ranged from 94 to 126 in trial 1, 99 to 114 in trial 2, and 112 to 138 in trial 3. Excretion patterns of the indicator were established by collecting "grab" fecal samples every 2 hours for a period of 5 days. A diurnal excretion pattern for chromium oxide was demonstrated and this pattern varied between animals on either regimen, *i.e.*, grazing or hand-fed herbage, within one trial and among the trials. Estimates of fecal production varied from 20 percent under-estimation to 49 percent over-estimation in trial 1, from minus 22 percent to plus 47 percent and from minus 34 percent to plus 54 percent of measured values in trials 2 and 3, respectively.

Although preliminary estimates using chromium oxide were not encouraging, efforts were continued with emphasis on techniques designed to improve recovery of the indicator. It was recognized that more frequent dosing of the indicator would be helpful. Efficacy of three different means of administering Cr_2O_3 twice daily to steers fed rush-sedge meadow hay was studied. The average percentage fecal recoveries of Cr_2O_3 were 84, 73, and 81 with cottonseed meal as a carrier, with "free" Cr_2O_3 in capsules, and Cr_2O_3 plus cellulose in capsules, respectively. Estimates of fecal production from samples collected at 8 a.m. and 4 p.m. for three respective methods were 119 percent, 138 percent and 123 percent of measured outputs. A test conducted to compare steers on limited-feed vs. steers on full-feed indicated that the quantity of forage consumed did not affect the estimates.

Data comparing various chromium oxide carriers used at the Oregon Station are presented in table 11. Encouraging results with chromium oxide used in a mixture with cellulose led to a further trial to investigate this means of supplying the indicator for estimating the fecal production of grazing steers. Chromic oxide recovery was essentially 100 percent when this technique was applied on the range and fecal production was estimated within 0.05 kilogram per day of actual measured values. The technique was in-

vestigated with steers grazing range alone and with similar steers supplemented with 0.22 kilogram barley per day. The results are shown in table 12. In three other trials with steers grazing range forage, similar Cr_2O_3 recoveries were experienced and fecal dry matter was estimated within an average of 0.45 kilogram per day for the three trials. Variations between trials ranged from a minus 0.44 to a plus 0.27 kilogram between actual and estimated daily fecal production (table 13).

Table 11. Comparison of three methods of administering Cr_2O_3 to estimate fecal output

Treatment	Steer	Fecal output (dry matter/day)		Estimated fecal output
		Actual	Estimated	actual
		kg	kg	%
Cottonseed meal: Cr_2O_3	1	4.0	5.0	124
	2	4.7	5.4	114
	Mean	4.35	5.2	119
"Free" Cr_2O_3 in capsules	3	3.7	4.7	126
	4	2.8	4.2	150
	Mean	3.25	4.45	138
Cellulose+: Cr_2O_3 in capsules	5	3.7	4.7	127
	6	3.2	3.8	119
	Mean	3.45	4.25	123

+ Solka Floc (The Brown Co., Berlin, N. H.).

Table 12. Comparison of estimated dry matter fecal outputs using Cr_2O_3 in cellulose carrier and total fecal collections with steers grazing Agropyron desertorum with and without barley supplement

Treatment	No. of animals	Mean actual fecal output	Mean estimated fecal output	Recovery Cr_2O_3
		kg	kg	%
No barley	6	3.84	3.79	102.2
Barley	6	4.20**	4.22*	99.6
LSD (5%)		0.21	0.40	7.8
LSD (1%)		0.28	0.54	10.5

* Significantly different ($P < 0.05$) from unsupplemented steers.

** Significantly different ($P < 0.01$) from unsupplemented steers.

Table 13. Average daily measured and estimated fecal output, Cr_2O_3 recovery and range of Cr_2O_3 recovery

Trial	Diet	No. of animals+	Actual fecal dry matter	Estimated fecal dry matter	Recovery Cr_2O_3	Range of Cr_2O_3 recovery
			kg/day	kg/day	%	%
1	Crested wheatgrass 0.68 kg CSM, 0.22 barley	6	3.63	3.18	103.7	91.9—111.7
2	Same as trial 1	4	4.06	4.16	99.0	94.0—103.8
3	Native range forage only	6	3.21	3.46	97.4	88.4—102.1
Mean of 3 trials		16	3.58	3.53	100.2	88.4—111.7

+ Values are averages of animals in each trial.

The possibilities of foliar application of Cr_2O_3 were investigated by using five steers in a total collection digestion trial on crested wheatgrass range. The trials were conducted during the first part of August on an area that had not been grazed that season. A 3-acre plot of crested wheatgrass was sprayed with Cr_2O_3 and a polyethylene adhesive material[1] with a wetting agent in water solution. The weight of dry forage per acre was estimated and Cr_2O_3 was applied to make up 0.3 to 0.7 percent of the dry weight of the forage. However, percent of ground cover, or density of the grass stand, was overestimated and the Cr_2O_3 content of the forage was actually less than 0.1 percent. This is below the desirable concentration for Cr_2O_3 when used for an indicator. The spraying was not difficult and the material adhered well to the foliage. The concentration of Cr_2O_3, while below the expected, was very uniform across the plot.

Digestion trials were conducted with a 7-day preliminary period and a 5-day collection period. Total fecal collections were made. Forage samples during the collection period were obtained by both clipping and by the rumen evacuation method. Samples obtained by both methods were similar with respect to Cr_2O_3 and nitrogen; therefore the average of all samples was used in calculating digestibility. The apparent dry matter and nitrogen digest-

[1] "plyac," a non-ionic spreader sticker by Allied Chemical Company

ibility for each animal is shown in table 14. Average daily fecal output and calculated intake are also presented.

This technique may be applicable under some conditions but needs further investigations. If the grass stand is adequate to give good ground cover it could be practical but even then may be limited to forage after growth has stopped. If applied during the "fast growth" part of the season a dilution factor for the Cr_2O_3 consistent with the increase of total forage would need to be calculated daily.

Table 14. Apparent dry matter and nitrogen digestibility, daily fecal dry matter and dry matter intake for each animal, calculated with Cr_2O_3 foliar application

Animal number	Apparent digestion coefficients		Daily fecal dry matter	Daily dry matter intake
	Nitrogen	Dry matter		
	%	%	kg	kg
10	47.12	57.78	2.81	4.88
11	45.82	52.80	3.32	6.30
12	46.08	53.43	3.06	5.75
16	41.35	52.78	2.42	4.59
17	39.59	51.36	3.28	6.39
Mean	43.99	53.63	2.98	5.58

Estimating Fecal Output and Dry Matter Intake

Chromium oxide

In conjunction with the Cr_2O_3 recovery studies reported under the digestibility section, Oregon workers measured forage intake of grazing animals at three different times during the grazing season with various levels of supplementation. Supplemental treatments were: (1) range forage alone with no supplementation, (2) range forage and supplemental levels calculated to give 0.91 kilogram daily gain, and (3) range forage and supplemental levels calculated to give 1.14 kilograms daily gain. Measured quantities of Cr_2O_3, dispersed on cellulose within a gelatin capsule were administered twice daily at 8 a.m. and 3 p.m. throughout a 7-day preliminary and a 5-day collection period. Each animal received 5 grams of Cr_2O_3 at each administration. Fecal grab samples were taken at the same time the Cr_2O_3 was given during the 5-day col-

Table 15. Body weight, daily gain, total intake, supplemental intake, and forage intake of yearling heifers on crested wheatgrass, calculated with the Cr_2O_3 technique

Date Treatment	Number of animals	Body weight	Daily gain	Total intake[+]	Supplement[+]	Forage intake[+]
		kg	kg	kg	kg	kg
6/16 — 6/20						
No supplements	3	290	0.84	5.82	—	5.82
Low supplements	3	290	0.88	7.18	0.34	6.84
High supplements	3	290	0.92	7.80	0.66	7.27
Mean	9	290	0.88	6.93	—	6.64
7/15 — 7/19						
No supplements	3	310	0.71	11.16	—	11.16
Low supplements	3	310	0.80	10.85	0.59	10.26
High supplements	3	311	0.73	9.35	0.86	8.49
Mean	9	310	0.74	10.46	—	9.97
8/15 — 8/19						
No supplements	3	327	0.60	10.09	—	10.09
Low supplements	3	335	1.04	11.56	1.23	10.33
High supplements	3	335	0.84	10.41	1.66	8.75
Mean	9	333	0.83	10.68	—	9.72

[+] Supplements were equal parts of rolled barley and cottonseed meal; all intake values are on a dry-matter basis.

lection period. Three animals were used on each level of supplementation.

Total intake and forage intake (total minus supplemental intake) appear in table 15, with the weight and daily gain of each animal during the trial. The most striking difference in intake appears between the first two periods of grazing. In general, when digestible nutrients in the forage at the various dates are taken into consideration, the calculated intake agrees with rate of gain of the animal. High levels of supplementation will inhibit the intake of low quality mature forage. The gain data at different levels of supplementation substantiate this supposition.

Lignin ratio

New Mexico workers (Hill *et al.*, 1961) compared the recovery of ingested lignin by confined heifers by using five procedures for collecting feces: (1) 24-hour fecal bag collection, (2) 4-hour fecal bag collection, (3) 6-hour fecal bag collection, (4) 4 hours on a

concrete platform and (5) 6 hours on a concrete platform. Lignin was determined by the 72 percent H_2SO_4 method. Percentage lignin recoveries for the five procedures, respectively, were: 117.4, 104.9, 105.7, 79.6, and 75.5. Calculations using dry matter excretion estimated by the 4-hour collection with fecal bags (multiplied by 6 for 24-hour collection) and by the lignin content of feed and feces resulted in an estimated dry matter intake of 7.18 kilograms as compared to an actual intake of 6.82 kilograms. Part of the high recovery of lignin using 24-hour fecal bag collection is probably due to sampling. Although all heifers were fed from a common source, a sample for analysis was obtained for each collection group, and the lignin content of the sample for the 24-hour collection heifers was considerably lower than the samples for others. Also, the procedures may have resulted in increased excretion of feces during the short test period.

New Mexico (Hill, 1965) also used lignin as an indicator of digestibility and 4 or 8-hour fecal collections, by means of harness and bag, to measure fecal output of cows on desert rangeland. The forage sample was hand-plucked by a technician while observing a cow during the daylight hours of a 24-hour grazing observation period. There were no significant differences among two cows within each breed (Hereford and Santa Gertrudis) nor among samples within cows. Digestibility estimates of Hereford cows grazing appeared satisfactory; however, this method did not provide reliable estimates of the organic matter intake of Santa Gertrudis cows because the estimates of fecal output were too low. These cows were very active and considerable difficulty occurred in catching and restraining them which resulted in fecal losses both before and after the collection bags were attached.

The lignin content of the forage was consistently higher in samples collected through a rumen fistula than in hand-plucked samples. Because lignin was used as the internal indicator, all digestibility values based on the lignin content of rumen samples were abnormally low. Thus, organic matter intakes based on rumen samples were unreliable as they considerably underestimated the organic matter intake necessary to support the cows.

Organic matter digestibility of range forage was similar among breeds when calculated by using the lignin-ratio method with samples of hand-plucked forage and collecting feces for 4 or 8-hour periods with harnesses and bags (table 16).

Table 16. Organic matter intake and digestibility by Hereford and Santa Gertrudis cows

Item	Breed and cow number			
	Hereford		Santa Gertrudis	
	1	2	3	4
Organic matter intake,				
Hand-plucked sample, g[a]	8,552	8,596	5,906	5,949
Rumen sample, g[b]	4,398	4,336	3,322	3,231
Organic matter digestibility				
Hand-plucked sample, %[c]	60.49	56.83	60.32	61.88
Rumen sample, %[b]	27.90	26.74	26.56	32.74

Statistical analysis

[a] Breeds **; cows within breeds, NS; Sample within cows, NS.

[b] Breeds * ; cows within breeds, NS; Sample within cows, NS.

[c] All comparisons, non-significant.

Table 17. Dry matter intake of forage at different stages of maturity by sheep as determined by two methods of lignin determination

Stage of maturity	Method	
	Ellis, et al., 1946	Van Soest, 1962
	kg/head/day	kg/head/day
Early (7/18 - 7/24)	1.07	1.70
Intermediate (8/3 - 8/9)	1.16	1.78
Late (8/19 - 8/25)	1.32	2.08

Workers from the U. S. Sheep Station at Dubois, Idaho (Price *et al.*, 1964) used the lignin-ratio technique for estimating dry matter intake and total fecal excretion. These workers compared values obtained by the 72 percent sulphuric acid-lignin method and by the ADL method of Van Soest (1963). Table 17 shows the dry matter intake values obtained by these two methods from forage at different stages of maturity.

In vitro

An *in vitro* technique for determining fecal output and dry matter intake of grazing animals has been described by workers at the California Station which eliminates the necessity of assuming the complete indigestibility of a naturally-occurring indicator. The new procedure was presented by Van Dyne and Meyer

(1964a) and involves (1) determining the digestibility of range forage and a "standard" forage in the artificial rumen or by the nylon bag procedure using inocula from animals grazing the range forage; (2) predicting the digestion of range forage by regression from the artificial rumen or nylon bag adjusted to the digestibility of the standard forage; and (3) use of the predicted digestibility, the composition of the forage, and the fecal output to determine forage intake. The method is illustrated in table 18. The forage intake of cattle and sheep grazing dry annual summer range predicted by various methods is shown in table 19. The values are

Table 18. Example of the procedure for estimating forage intake from in vitro digestibility estimates+

Required information		
(1)	Forage cellulose, %	42.64
(2)	Fecal cellulose, %	34.37
(3)	Fecal output, kg/day	3.03
(4)	In vitro digestibility of cellulose of range forage using range diet inocula, %	61.4
(5)	In vitro digestibility of cellulose of standard forage using range diet inocula, %	58.5
(6)	In vitro digestibility of cellulose of standard forage using standard forage diet inocula, %	54.5
Calculated results		
(7)	Adjusted in vitro digestibility of cellulose in range forage, % $\frac{(4)}{(5)}\ (6) = \frac{(61.4)}{(58.5)}\ (54.5) =$	57.2
(8)	Predicted in vivo digestibility of cellulose in range forage by grazing animals, % $59.6 - 0.11(7) = 59.6 - (0.11)\ (57.2) =$	53.3
(9)	Predicted forage intake, kg/day $\frac{(100)\ (3)\ (2)}{(100)\ (1) - (1)\ (8)} \quad \frac{(100)\ (3.03)\ (34.37)}{(100)\ (42.64) - (42.64)\ (53.3)} =$	5.23

+ For development of the theoretical considerations see Van Dyne and Meyer (1964a).

Table 19. Comparison of forage dry matter intake estimated by various methods+

Method of prediction	Cattle	Sheep
	kg/head/day	
Lignin ratio and total fecal collection	5.1	0.82
Predicted from artificial rumen or nylon bag digestibility estimates:		
Cellulose in artificial rumen	5.0	0.86
Cellulose in nylon bag	5.1	0.86
Dry matter in nylon bag	5.9	0.91

+ Modified from Van Dyne and Meyer (1964b).

the means of three determinations made in early, mid-, and late summer. The estimate forage consumption by cellulose digestion either in the artificial rumen or nylon bag agrees well with that estimated from lignin ratio.

The method of estimating forage intake from *in vitro* digestibility estimates has the advantage of being applicable to all stages of forage maturity while those based on chromogens or lignin are not always accurate at all stages. On the other hand the *in vitro* procedure requires more effort than the lignin ratio technique. Both methods require an accurate sample of grazed forage and accurate estimation of fecal output.

Total Fecal Collection Under Grazing Conditions

Total fecal collections have been employed quite extensively by Idaho, Nevada, Oregon, Utah, New Mexico, and Washington workers. The apparatus is essentially the same at each location (Cook *et al.*, 1952; Gorski *et al.*, 1957; Erwin *et al.*, 1959; and Lesperance and Bohman, 1961).

Probably the one most important item regarding digestion studies with range animals is to select and train the animals properly. Animals just "harnessed" up and turned loose will not consume range feed in a normal manner. Consistent and normal consumption is most important to take out as much variation in digestibility data as possible. If animals are trained, and the nervous ones culled, the digestibility data will have much less variation.

Total fecal collection work has been conducted at the U. S. Sheep Station at Dubois, Idaho. In their work "fecal collection animals" (approximately the same body weight as fistula animals) graze with the "forage sampling animals" during a digestion trial. Canvas zippered bags are attached to the animals several days before a digestion trial. Much time and care is spent adjusting bags to each individual animal so that no feces will be lost and to ensure that the straps are not making the animal uncomfortable. The fecal bags remain on the animals for the length of the digestion trial. The collection bags are emptied twice daily. Price *et al.* (1964), under controlled conditions with measured individual feed consumption, compared the feed intake of bagged versus non-

bagged animals and did not find any significant difference between the two groups.

An apparatus for collecting both feces and urine from range cattle was developed by Nevada workers (Lesperance and Bohman, 1961). This equipment consists of an inflatable urethral catheter connected to a urine-collecting container carried on the back of the animal and a vinyl-coated fecal collecting bag.

IN VITRO AND IN VIVO METHODS FOR ESTIMATING POTENTIAL NUTRITIVE VALUE OR DIGESTIBILITY OF RANGE FORAGE

Summary

The nylon bag and artificial rumen techniques can be used to detect differences in forage quality. Based on results obtained by using the artificial rumen in the western region, the following conditions should be observed:

1. If possible, obtain inocula from a donor animal consuming the same forage as being tested in the artificial rumen.
2. Strained rumen liquor is satisfactory.
3. A 24-hour fermentation period will measure *in vivo* cellulose digestion adequately.

In using the nylon bag technique the following conditions should be observed:

1. Fineness of grind for the sample — 2 millimeter mesh Wiley mill screen appears adequate.
2. Forty-eight hour fermentation period.
3. Incubate samples in animals consuming the same forages as being tested in the nylon bag.

To estimate the nutritive value of range forage, it would be desirable to use esophageal-fistulated or rumen-fistulated animals as forage samplers. The digestibility of the grazed vegetation could be determined with nylon bags suspended in rumen-fistulated animals grazing the same vegetation as the samplers, or by artificial rumen techniques using inoculum from animals grazing the same vegetation.

Introduction

The rangelands of the western United States produce a variety of forages in such sparse amounts that it is practically impossible to obtain sufficient forage to determine digestibility by conventional means. Many investigators have studied the influence of environmental factors on the chemical analyses of clipped range forages. However, this method does not indicate the nutritive value of the forage to livestock. In recent years the artificial rumen and nylon bag techniques have been used to evaluate small forage samples.

Artificial Rumen Technique

California workers have adapted the artificial rumen technique for use in range studies with large numbers of samples. The system described by Van Dyne (1963) has a capacity of 170, 100-milliliter centrifuge tubes. The system required only simple equipment for this operation, thus facilitating its use in field laboratories.

Source of inoculum

The influence of source of inoculum on artificial rumen fermentation has been studied by California, Colorado and Wyoming investigators. At California the cellulose digestibility of range forage was higher when the innoculum came from animals fed alfalfa compared with inoculum from animals eating the same forage being tested in the artificial rumen (Van Dyne and Weir, 1966). As shown in table 20, *in vitro* cellulose digestion was 56.7 percent and 56.2 percent when inoculum was obtained from cattle and sheep fed alfalfa hay respectively versus 52.2 percent and 51.7 percent when inoculum was obtained from cattle and sheep grazing the test forage. Inoculum from cattle or sheep grazing dry range forage was similar.

In another adaptation from the Van Dyne and Weir (1966) study, Van Dyne and Meyer (1964b) reported that inocula from cattle digested less alfalfa cellulose *in vitro* than inocula from sheep when both were fed alfalfa hay. The reverse of this was true when the inocula was obtained from sheep and cattle grazing range forages. When the donor animal was fed alfalfa hay, Van Dyne (1962)

Table 20. Range forage cellulose digestibility in the artificial rumen using different sources of inoculum

Source of inoculum+	Cellulose digestion
	%
Cattle:	
Grazing range forage	52.2
Fed alfalfa hay	56.7
Mean	54.5
Sheep:	
Grazing range forage	51.7
Fed alfalfa hay	56.2
Mean	54.0

+ Adapted from Van Dyne and Weir (1966).

found higher *in vitro* cellulose digestion compared with inoculum from an animal fed oat hay. He questioned the validity of using inoculum from an animal fed a different kind of feed than the one being evaluated *in vitro*.

Hoehne (1963) reported that *in vitro* volatile fatty acid (VFA) production using fresh grass inoculum was similar to VFA production *in vivo*, whereas there was a significant difference between *in vitro* VFA production and *in vivo* VFA production using dried grass inoculum.

Eikenberry (1963) reported that the source of inoculum significantly influenced cellulose and dry matter digestion in the artificial rumen. He compared inoculum from steers fed good quality first-cutting alfalfa hay, poor quality crested wheatgrass hay, good quality sedge-type flood meadow hay, and fair quality oat regrowth cut for hay.

Length of fermentation

Van Dyne (1962) compared 12, 24, 48 hour *in vitro* fermentation periods. He found that cellulose digestion of different forages increased with time, but the forages did not change relative positions in cellulose digestibility between 24 and 48 hours. Using inocula from a steer fed native meadow hay, Wallace *et al.* (1965) found that 24-hour *in vitro* fermentation underestimated *in vivo* digestion in the sheep, while 48-hour fermentation overestimated animal digestion (see table 21).

Van Dyne (1962) reported that the method of processing the inocula significantly influenced *in vitro* cellulose digestion. Cellulose digestion was similar when strained rumen fluid and a phosphate buffer extracted fluid were used, however, a centrifuged

Table 21. Cellulose digestion of meadow hay+

Harvest date	24-hour digestion	48-hour digestion	In vivo sheep digestibility
	%	%	%
June 9	64.6	79.2	68.0
June 28	53.7	70.9	59.8
July 17	49.0	67.0	55.2
August 4	48.6	66.9	54.0

+ Wallace et al. (1965).

suspension of rumen microorganisms resulted in considerably less cellulose digestion.

Trei *et al.* (1963) compared *in vitro* cellulose digestion of solka floc, alfalfa, coastal Bermuda (*Cynodon spp.*) and blue panic (*Panicum antidotal*) hays after 3, 6, 12, 24, and 48 hours fermentation. Cellulose digestion was similar between the grasses and solka floc until the end of the 12-hour fermentation period. However, at 12 hours the cellulose digestion of solka floc was greater than cellulose digestion of the grasses, but it was not digested to the same extent as alfalfa cellulose. *In vitro* cellulose digestibilities of alfalfa, solka floc, Bermuda and panic hays differed significantly.

Van Dyne and Meyer (1964a) have suggested that the artificial rumen be used to estimate cellulose digestibility of forage samples collected via an esophageal fistula. The inoculum for the artificial rumen should come from an animal grazing the same forage. By using regression equations, the macrodigestion of the cellulose in the range forage can be predicted from the *in vitro* cellulose digestibility measurements. These estimates of digestibility are combined with estimates of fecal production to predict forage intake.

Influence of stage of maturity on in vitro digestibility

The Oregon and Arizona investigators have studied the influence of stage of maturity on *in vitro* cellulose digestion. The cellulose in coastal Bermuda and blue panic hays cut at two stages of maturity (June 22 and August 17) was digested similarly in the artificial rumen (Trei *et al.*, 1963). Samples of bluebunch wheatgrass (*Agropyron spicatum*), squirreltail (*Sitanion hystrix*), Idaho fescue (*Festuca idahoenis*), Junegrass (*Koeleria cristata*), Thurber's needlegrass (*Stipa thurberiana*), and crested wheatgrass (*Agropyron desertorum*) were collected on six dates (April 30 to July 15) in 1959 and on five dates (May 11 to August 5) in 1960 by Wallace *et al.* (1961). *In vitro* cellulose digestibility of these range grasses was not materially influenced by date of sampling until after June 2 (table 22). After this date cellulose digestibility generally decreased at each sampling date. The mean cellulose content of six range grasses increased from 23.9 percent at the first clipping to 30.5 percent at the last clipping. The corresponding crude protein contents were 19.0 and 5.8 percent.

Table 22. In vitro cellulose digestibility of various range grasses as influenced by date of sampling during two summer periods+

Year & date of sampling	Specie[a]						
	Idaho fescue	Bluebunch wheatgrass	Squirrel-tail	Thurber's needlegrass	Crested wheatgrass	June-grass	Mean[b]
	%	%	%	%	%	%	
1959							
4/30	62.0	72.6	72.2	68.7	76.3	74.0	71.0[c]
5/18	65.2	66.6	69.2	68.5	68.3	71.2	68.2[c]
6/2	57.3	62.4	74.0	66.8	68.3	69.9	66.4[c]
6/16	47.2	55.2	59.4	61.5	72.2	66.4	60.3[d]
7/1	45.5	46.5	52.3	57.2	53.0	61.4	52.6[e]
7/15	52.0	37.2	62.6	62.7	48.0	60.7	53.9[e]
Mean[b]	54.9[e]	56.7[e]	65.0[d]	64.2[d]	64.3[d]	67.3[d]	
1960							
5/11	64.7	71.8	70.0	65.6	74.0	76.8	70.5[c]
5/23	61.4	66.1	70.9	71.8	69.7	71.2	68.5[c]
6/2	61.7	70.5	71.9	71.4	73.3	76.8	70.9[c]
6/16	45.4	50.5	58.7	59.4	65.0	58.3	62.2[d]
8/5	45.6	43.7	52.2	57.9	48.1	63.6	51.8[e]
Mean[b]	55.8[d]	60.5[cd]	64.7[ce]	65.2[ce]	66.0[e]	69.3[e]	

+ Wallace et al. (1961).
[a] All values are the average of two determinations.
[b cde] Means having the same superscript letters are not significantly different ($P < .05$).

Hay samples from native meadows were collected at six dates by Wallace *et al.* (1961). *In vitro* cellulose digestion of the native hay increased when date of harvest was delayed from May 4 until May 18 and then declined with each later date of harvest up to July 13, the last harvest date.

In vitro vs. in vivo digestibility

Arizona investigators suggested that *in vitro* cellulose digestion may be of doubtful value in comparing the relative intake and energy digestibility of varieties within a specie. Moapa and Lahontan alfalfa hays were harvested at three stages of maturity (mid-bud, early and full bloom). The *in vitro* cellulose digestion values did not reflect the lower *ad libitum* intake or lower digestibility of the Lahontan mid-bud cutting that was observed *in vivo*. The 12-hour digestion of cellulose was correlated with feed intake, whereas 24-hour digestion was correlated with *in vivo* digestibility.

Taylor *et al.* (1960) reported significantly lower digestibility of the cellulose in blue gramma and Sudan grass hays with a 48-hour *in vitro* fermentation than with conventional *in vivo* digesti-

bility with sheep. However, a small difference between *in vivo* and *in vitro* cellulose digestion of alfalfa hay was not significant. Rumen liquor for all *in vitro* studies was obtained from sheep fed alfalfa hay. The standard deviations were lower for the *in vitro* digestibilities.

Trei *et al.* (1963) used the relative intake and digestible energy values to calculate estimates of the nutritive value index (NVI) for alfalfa, coastal Bermuda and blue panic hays (see table 23). NVI was defined as the relative intake times the percent digestible energy. Simple correlations of 0.95 were found when the NVI of each hay was correlated with either the 12-hour *in vitro* cellulose digestion or the 24-hour accumulative area under the fermentation curve. The product of the 12- and 24-hour cellulose digestion values was also highly correlated with NVI ($r = 0.92$).

Arizona workers calculated simple correlations between 12-hour *in vitro* cellulose digestion values and the crude protein and phosphorus content of range forages. Within a range forage specie, there was a high relationship between the *in vitro* cellulose digestion and crude protein or phosphorus content of the plant.

Eikenberry (1963) did not find a significant correlation of cellulose and dry matter digestibility of alfalfa, crested wheatgrass, and oat hays in the artificial rumen with digestibility in steers using total collection methods. However, artificial rumen and conventional digestibility determinations did rank the forages in the same order.

Table 23. In vitro and in vivo measurements used in calculations of the in vitro index and effective nutritive value index+

Forage	In vitro			In vivo		
	12-hr[a]	24-hr[a]	IVI[b]	RI[c]	DE[d]	NVI[e]
	%	%			%	
Alfalfa	45.0	56.5	25.4	109.0	62.2	68.2
Blue panic (June)	3.8	11.0	4.2	69.4	53.3	37.0
Blue panic (August)	5.8	10.6	6.1	53.2	51.2	27.2
Coastal Bermuda (June)	23.8	42.5	10.1	91.5	51.6	47.2
Coastal Bermuda (August)	20.9	48.1	10.1	81.1	56.6	45.9

+ Trei et al. (1963).

[a] In vitro cellulose digestion.

[b] $\frac{12 \times 24\text{-hour in vitro cellulose digestion values}}{100}$ = in vitro index (IVI).

[c] Relative intake values as proposed by Crampton et al. (1960).

[d] Digestible energy.

[e] Nutritive value index (NVI) = relative intake x digestible energy (percent).

Nylon Bag Technique

Sample preparation and fermentation time

Elliston (1961) described in detail the nylon bag technique used at Arizona. He and Gallinger (1965) both reported that fineness of sample grind did not significantly influence dry matter disappearance from the nylon bag. Both investigators reported that significantly more dry matter disappeared with 48 hours incubation compared with 24 hours. Gallinger (1965) found no difference in dry and organic matter disappearance when he compared 72 hours incubation with 48 hours (table 24).

Van Dyne (1962) reported that cellulose digestion in the nylon bag was inversely related to sample size (2 to 10 grams) and directly related to fermentation time (24 to 72 hours). Sheep could digest cellulose and dry matter in cattle-grazed forage as well as cattle and cattle could digest sheep-grazed forage as well as sheep. Compared with light rinsing, exhaustive rinsing of the nylon bags after removal from the rumen resulted in significantly higher dry matter (64 percent vs. 53 percent) and cellulose (62 percent vs. 58 percent) digestibilities. These differences were greater for dry matter than for cellulose.

Gallinger and Kercher (1964) reported that approximately 1 percent of the dry matter was lost from the nylon bags when they were agitated in a warm water bath for 72 hours. Most of this loss was inorganic. Elliston (1961) reported a 2.5 percent dry matter loss when solka floc was suspended in nylon bags in water for 72 hours.

Table 24. Mean digestion coefficients among roughages and length of fermentation periods for dry matter†

Roughage	Fermentation periods			
	24 hours	48 hours	72 hours	Mean §
	%	%	%	%
Alfalfa	39.3	41.8	39.0	40.0[a]
Native	21.1	29.9	33.2	28.0[b]
Straw	7.2	11.7	11.4	10.1[c]
Mean[a]	22.5[a]	27.8[b]	27.8[b]	

† Gallinger (1965).
§ Means having the same superscript (a, b, c,) are not significantly different ($P < .05$).

The Oregon investigators have attempted to pass nylon bags of forage through the intact digestive tract of steers. In seven trials they have been unable to recover the bags from the feces, litter, feed refusals, or rumen contents.

Influence of diet on nylon bag digestion

Arizona, California and Wyoming workers found that the diet of the fistulated animal influenced digestion in the nylon bag. Significant differences were found between cellulose digestion of forage samples in nylon bags between rumen-fistulated cattle and sheep when they grazed together on the range but not when they were fed hay (Van Dyne, 1962; Van Dyne and Weir (1966).

Significantly less dry and organic matter disappeared from nylon bags suspended in the rumen of steers fed 75 percent chopped alfalfa hay — 25 percent barley compared with 100 percent alfalfa hay (23.4 percent vs. 28.8 percent for dry matter), according to Gallinger and Kercher (1964). The California investigators (Van Dyne and Weir, 1966) also found that cellulose digestibility of range forage in nylon bags was higher when they were incubated in animals fed alfalfa hay compared with animals grazing the same forage as in the nylon bag (table 25).

The nylon bag technique was able to detect differences in dry matter disappearances among forages. Elliston (1961) reported that the feed sample in the bag accounted for 87.4 percent of the variation in dry matter disappearance when variations resulting from animals, rations, fineness of grind, and time of incu-

Table 25. Range forage cellulose digestibility in the nylon bag using different sources of innoculum+

Source of inoculum	Cellulose digestion
	%
Cattle	
Grazing range forage	53.5
Fed alfalfa hay	57.8
Mean	55.7
Sheep	
Grazing range forage	55.7
Fed alfalfa hay	58.7
Mean	57.2

+ Adapted from Van Dyne and Weir (1966).

bation were considered (24 vs. 48 hours). Gallinger (1965) found significantly more dry and organic matter disappeared from alfalfa hay than from native hay or wheat straw when fermented in the nylon bag. Significantly more dry and organic matter disappeared from native hay than from wheat straw. There also was an interaction of fermentation time and type of roughage in the bag ($P < .05$). Dry and organic matter disappearance from alfalfa hay was similar at 24, 48, or 72 hours, whereas the disappearance of these nutrients increased as fermentation time increased for native hay and wheat straw. The interaction of type of roughage in the nylon bag and the ration fed to the fistulated animal was also significant ($P < .05$). Reduction in dry and organic matter disappearance from native hay and wheat straw was greater when they were suspended in steers fed 75 percent alfalfa — 25 percent barley than with these forages suspended in steers fed 100 percent alfalfa.

Gallinger (1965) collected three grass and four browse species at three locations in Wyoming in the spring and fall. These were incubated for 48 hours in the rumen of steers fed native hay. There were significant differences in the digestibility of the forages (needle-and-thread, *Stipa comata* Indian ricegrass, *Orzopsis hymenoides;* crested wheatgrass, *Agropyron cristatum;* shadscale, *Atriplex confertifolia;* winterfat, *Eurotia lanata;* big sage, *Artemisia tridentata;* and saltbush, *Atriplex gardernii*) used in this study when measured by the nylon bag technique. Dry and organic matter digestibility in the nylon bag was higher for plants collected in the spring compared with those collected in the fall (table

Table 26. Mean dry matter disappearance between spring and fall harvested range forages+

Forages	Means §	
	Spring	Fall
	%	%
Stipa comata (needle-and-thread)	42.6^{d}	37.3^{e}
Oryzopsis hymenoides (Indian rice grass)	45.7^{bc}	35.2^{f}
Agropyron cristatum (crested wheatgrass)	41.6^{d}	32.8^{g}
Atriplex gardernii (saltbush)	47.2^{ab}	37.8^{e}
Atriplex confertifolia (shadscale)	47.5^{a}	30.2^{h}
Eurotia lanata (winterfat)	42.4^{d}	22.3^{i}
Artemisia tridentata (big sage)	46.0^{bc}	35.2^{f}
Mean	44.7	33.0

+ Gallinger (1965).

§ Means with the same superscript (a, b, c, d, e, f, g, h, i,) are not significantly different ($P < .05$).

26). The interaction between species of plant and season of collection was significant. Browse species fluctuated more seasonally than grasses. Nutrient disappearance from the nylon bag differed significantly depending on the site of collection. The interaction of site of collection and species of plant significantly influenced dry and organic matter digestibility. The interaction of site of collection and season also significantly influenced dry and organic matter digestibility.

Nylon bag digestion vs. in vivo digestibility

Elliston (1961) conducted a steer digestion trial by using alfalfa, blue panic hay and Bermuda straw concurrently with a nylon bag study of the same feeds with incubation times of 8, 24, 48, 72 and 96 hours. Although there was a recognizable difference between samples in the dry matter disappearance rates from the nylon bags (which paralleled results obtained in the digestion trial), no specific hour of incubation could be selected to best correlate with conventional digestibility of various roughages. Howard (1963) found a strong inverse linear negative relationship between lignin content and dry matter loss from samples incubated in the nylon bag for 24 hours.

California investigators found that nylon bag estimates of cellulose digestibility of range forages were slightly but consistently higher than those obtained from the artificial rumen.

Eikenberry (1963) found that the correlations between nylon bag estimates of cellulose and dry matter digestibility and digestibility determined by total collection methods with steers fed alfalfa, oat, or native crested wheatgrass hays were not significant. Both methods detected significant differences in cellulose and dry matter digestibilities of the three hays. Kercher *et al.* (1964) reported no significant correlations between the digestion of nine native hay samples in nylon bags suspended in fistulated steers fed native hay with digestion of the same hays in intact steers using total collection methods.

Arizona workers suggested that *in vivo* digestibility of dry matter and cellulose, dry matter consumption, total rumen volatile fatty acid concentration, nylon bag dry matter digestibility, and *in vitro* cellulose digestibility were all effective in evaluating alfalfa hay, a high quality forage vs. Bermuda straw, a low quality

forage. Correlations, however, were not established between methods.

Van Dyne and Weir (1964a) reported that alfalfa hay, solka floc and six forages collected by esophageal-fistulated sheep and cattle were not ranked in the same order by artificial rumen-cellulose digestion, nylon bag-cellulose digestion, or nylon bag-dry matter digestion. Cellulose digestibility was higher in forage samples collected by cattle with esophageal fistulas when compared with sheep. Forage samples collected by esophageal-fistulated animals were digested better in the nylon bag than hand-clipped forage samples (table 27).

When digestion was estimated by the lignin ratio technique, the correlation between microdigestion and macrodigestion estimates of dry matter and cellulose digestibility was about 0.72. The correlation was 0.45 for only the cellulose digestion estimates (Van Dyne and Weir, 1964a).

Van Dyne and Weir (1964b) reported no difference between cattle and sheep inocula in estimating *in vitro* and *in vivo* measures of digestion. Estimates of digestion were about one-half as variable when the animals providing the inoculum were kept in drylot on alfalfa hay instead of grazing the range. The nylon bag technique appeared to be more accurate but more variable than the artificial rumen technique for evaluating forage digestibility. There were more differences in digestive power among steers than among sheep. About six cattle and four sheep would be required as inocula sources to estimate microdigestion within 10 percent of the mean with 95 percent confidence. More animals would be required for the same precision by macrodigestion techniques.

Table 27. Digestion of five clipped grasses and mixed forages collected by cattle and sheep (nylon bag digestion)+

Species	Cellulose digestion	
	Cattle	Sheep
	%	%
Slender oat, **Avena barbata**	53	52
Soft brome, **Bromus mollis**	46	48
Ripgut grass, **Bromus rigidus**	48	46
Foxtail chess, **Bromus rubens**	43	48
California needlegrass, **Stipa pulchra**	21	27
Mixed forage collected by esophageal fistula		
Sheep	51	56
Cattle	52	60

+ Van Dyne and Weir (1964).

NUTRIENT REQUIREMENTS AND RANGE SUPPLEMENTATION

Summary

The digestible energy (DE), metabolizable energy (ME) and total digestible nutrient (TDN) requirements of growing-finishing beef heifers can be expressed by the equations

$$\begin{aligned} DE &= 0.162W^{0.75}\ [1 + 1.85\ (gn)] \\ ME &= 0.143W^{0.75}\ [1 + 1.80\ (gn)] \\ \text{and } TDN &= 0.034W^{0.75}\ [1 + 1.85\ (gn)] \end{aligned}$$

when the ration consists of roughage. If a high concentrate ration is fed the respective equations are

$$\begin{aligned} DE &= 0.130W^{0.75}\ [1 + 1.30\ (gn)] \\ ME &= 0.110W^{0.75}\ [1 + 1.32\ (gn)] \\ \text{and } TDN &= 0.030W^{0.75}\ [1 + 1.34\ (gn)]. \end{aligned}$$

In each case W is body weight in kilograms and gn (daily weight gain) is in kilograms.

The net energy requirements for maintenance (NE_m) for both steer and heifers can be expressed

$$NE_m = 0.075W^{0.75}$$

and the net energy required for weight gain can be expressed

$$\begin{aligned} NE_{gn} &= 0.061W^{0.75} \text{ for steers and} \\ NE_{gn} &= 0.068W^{0.75} \text{ for heifers} \end{aligned}$$

where NE_m is in megacalories per day, NE_{gn} is megacalories per kilogram of daily weight gain and W is body weight in kilograms.

Range supplementation studies have shown that no blanket recommendations can be made to cover all conditions. The necessary composition of the supplement will vary depending upon the type of range and the season of supplementation. Data are presented on the effects of protein, energy, and phosphorus supplementation on different types of ranges and during different seasons.

Energy Requirements

Digestible and metabolizable energy

The California, Washington, and Wyoming Stations have studied the digestible energy (DE) and metabolizable energy

(ME) requirements for growth and for reproduction and lactation. The California studies have been concentrated on cattle during the growing period following weaning. Initial studies were conducted under dry lot conditions to work out techniques. Yearling heifers were fed either a high- or low-energy ration at three levels. Digestibility determinations made on the same rations provided data on their DE and ME content. The low-energy ration was made up entirely of hay while the high-energy ration contained 98 percent concentrates which provided wide extremes in energy level. Measurement of total feed consumption and energy content of the feed provided data on energy consumption. Liveweight gains were corrected to an empty body basis by the equation $Y = 31.8 + 1.45X$ where X is the hot carcass weight in kilograms. Energy retention was determined by the comparative slaughter method (Lofgreen, 1964). From the data on energy consumption, energy retention, and weight gains it is possible to determine the energy requirements for maintenance and gain. An equation of the type

$$x = aw^b[1 + k\ (gn)\ ]$$

was fitted to the data. In the equation x is the daily energy requirement (DE or ME) in megacalories, w is the mean empty body weight in kilograms and gn (daily weight gain) is in kilograms. The constants, a and k, were calculated from the data and the exponent, b, was assumed to be 0.75 (Kleiber, 1961). For example, for the data on DE intake and energy retained shown in table 28 it is possible to determine that a is equal to 0.162 megacalorie, for the high roughage ration and 0.130 megacalorie for the high concentrate ration. This means that on the high roughage ration 0.162 megacalorie of DE per kilogram of $W^{0.75}$ are required for maintenance (when $gn = 0$) but only 0.130 megacalorie are required on the high concentrate ration. Having the numerical value of a makes it possible to determine k. The values for k shown in table 28 were determined for each group of six heifers on the two higher levels of feeding using the mean values for a of 0.162 and 0.130 megacalorie for the high roughage and high concentrate rations respectively. The value of k was not calculated on the two low levels of feeding because of the inaccuracy of measuring the gain at these levels. Using this procedure, equations expressing the DE, ME, and TDN requirements for growing-finishing beef heifers have been determined and are shown in table 29. These equations illustrate the well-known fact that

Table 28. Determination of digestibile energy requirements

Level of feeding	Replication	Number of heifers	Mean $W^{0.75}$	Daily DE intake	Daily energy retained	DE required for maintenance (a)	Daily weight gain	DE k values
			kg	megcal	megcal	megcal $W^{0.75}$	kg	
High roughage ration								
Low	1	6	57.9	8.30	—0.04	0.162	—0.04	—
	2	6	57.9	9.73	—0.06	0.162	—0.04	—
	3	6	58.7	9.85	—0.03	0.162	—0.07	—
Medium	1	6	64.0	15.02	1.11	0.162	0.24	1.87
	2	6	64.2	14.64	1.04	0.162	0.25	1.63
	3	6	63.1	14.52	0.99	0.162	0.23	1.83
High	1	6	68.9	23.72	3.03	0.162	0.65	1.73
	2	6	67.7	24.18	2.86	0.162	0.59	2.04
	3	6	66.8	24.10	2.79	0.162	0.62	1.98
							High roughage mean	1.85
High concentrate ration								
Low	1	6	61.2	8.60	0.52	0.130	0.10	—
	2	6	61.6	9.71	0.83	0.130	0.16	—
	3	6	63.6	10.27	0.52	0.130	0.09	—
Medium	1	6	70.3	17.19	3.28	0.130	0.70	1.26
	2	6	66.6	15.78	2.20	0.130	0.74	1.11
	3	6	68.9	16.53	2.74	0.130	0.57	1.48
High	1	6	75.3	23.49	5.39	0.130	1.09	1.28
	2	6	75.2	24.69	5.65	0.130	1.13	1.35
	3	6	73.0	23.71	5.21	0.130	1.14	1.31
							High concentrate mean	1.30

it requires more DE, ME, or TDN from roughages than from concentrates to achieve a given level of performance. It is apparent that requirements determined for cattle fed concentrates may not accurately express the energy requirements of cattle grazing essentially a roughage diet. When combined with techniques to measure forage consumption these methods can be adapted to the determination of the energy requirements of grazing animals.

Workers at the Washington Station have initiated studies designed to determine the energy requirements of grazing sheep. The esophageal fistula method was used to sample the diet, grab samples of feces and urine were collected twice daily and dry matter digestibility and consumption determined by use of lignin as the internal indicator and chromic oxide as the external indicator (Kane *et al.*, 1953; Smith and Reid, 1955). Urinary output was calculated by a creatinine index method proposed by Butcher and Harris (1957). The equations describing the relationship of energy intake to performance are shown in table 30. For the purpose of comparison the equations of Garrett *et al.* (1959) are also shown. For a 25-kilogram lamb gaining 0.15 kilogram per day the DE requirement calculated from the two Washington equa-

Table 29. Energy requirements for growing finishing beef heifers+

High roughage	High concentrate
DE = $0.162W^{0.75}$ (1 + 1.85 gn)	DE = $0.130W^{0.75}$ [(1 + 1.30 (gn)]
ME = $0.143W^{0.75}$ (1 + 1.80 gn)	ME = $0.110W^{0.75}$ [(1 + 1.32 (gn)]
TDN = $0.034W^{0.75}$ (1 + 1.85 gn)	TDN = $0.030W^{0.75}$ [(1 + 1.34 (gn)]

+ DE and ME are in megacalories per day, TDN is in kilograms per day, W is in kilograms, and daily weight gain (gn) is in kilograms per day.

Table 30. Equations expressing the DE and ME requirements of sheep+

	Washington	Garrett, et al.
Trial I	DE = $93.6W^{0.73}$ (1 + 1.21 gn)	
	ME = $59.0W^{0.73}$ (1 + 1.57 gn)	DE = $138W^{0.75}$ [(1 + 5.3 (gn)]
Trial II	DE = $135.4W^{0.73}$ (1 + 0.94 gn)	ME = $112W^{0.75}$ [(1 + 5.5 (gn)]
	ME = $100.8W^{0.73}$ (1 + 1.01 gn)	

+ DE and ME are kilocalories per day, W is in kilograms and daily weight gain (gn) is in kilograms per day.

tions would be 1160 and 1621 kilocalories per day for trials I and II, respectively, while that calculated by the equation of Garrett is 2782 kilocalories per day. The equation developed in trial II agrees well with Garrett's equation for the maintenance requirement (1422 and 1546 kilocalories, respectively) but differs markedly in the requirement for the 0.15 kilogram daily gain (199 kilocalories and 1236 kilocalories, respectively).

Net energy

The California workers have determined the net energy (NE) requirements of growing-finishing beef heifers by use of the comparative slaughter method (Lofgreen, 1964) to determine energy retention. Although the studies have not yet been extended to grazing animals, requirements and feed values were determined on the same extremes of energy level used in the DE and ME trials discussed above.

In recognition of the fact that ME is used more efficiently for maintenance than for gain, a system has been developed which expressed NE requirements for gain separate from that required for maintenance. There are thus two NE values for requirements and feed values. One value (NE_m) expresses the NE requirement for maintenance [basal metabolism (NE_{bm}) and voluntary activity (NE_a)] and correspondingly the NE value of the feed for meeting the maintenance requirement. The other value (NE_p) expresses the NE requirement for production, *i.e.* (weight gain (NE_{gn}) reproduction (NE_r), lactation (NE_l), eggs (NE_e), or wool (NE_w); after the maintenance requirement has been satisfied and also the NE value of a feed for meeting the requirement for production above maintenance. The basic data upon which this system is based have been published (Lofgreen *et al.*, 1963; Lofgreen, 1964 and 1965). The NE_m is in megacalories per day and W is body weight in kilograms. In a manner similar to that described for DE, the constant, k, was found to be 0.88.

The comparative net energy requirements for steers and heifers were studied in a second trial. Figure 5 shows the heat production (HP) of steers and heifers fed the same basal ration with varying levels of grain fed to promote three rates of gain. The two lines representing the equations describing the relationship of heat production to metabolizable energy intake for the steers and heifers were indistinguishable. Energy equilibrium in both

cases could be maintained on a metabolizable energy intake of 120 kilocalories per kilogram of $W^{0.75}$. These data indicate, therefore, that there was no difference in the maintenance requirement for net energy of steers and heifers. The requirement can thus be expressed as $0.075W^{0.75}_{kg}$ megacalorie per day for both steers and heifers and the total net energy as

$$NE_{m+gn} = 0.75W^{0.75}_{kg} [1 + k (gn)].$$

Using the data in table 31, the values of k have been calculated for each group of six steers and heifers to compare the energy requirements for weight gain. The difference between 0.81, the k value for steers and 0.91, that for heifers is highly significant. The agreement between the k values for net energy obtained for the heifers in the first experiment (0.88) and the second (0.91) is good.

On the basis of these studies it appears that the NE require-

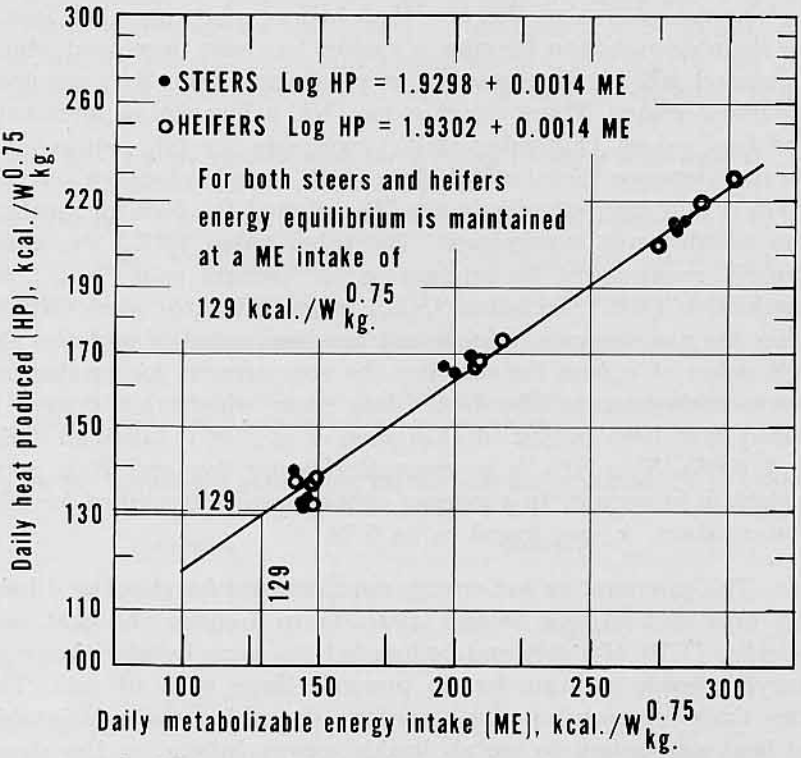


Figure 5. Relationship of heat production and metabolizable energy intake of steers and heifers.

ment for heifers is approximately 10 percent greater than for steers, a difference which is probably due to the higher fat content of heifer gains. Until further data are available it is proposed that the total net energy requirements of growing-finishing beef cattle may be expressed:

$$\text{Steers: } NE_{m+gn} = 0.075W^{0.75} [(1 + 0.81 (gn)]$$
$$\text{Heifers: } NE_{m+gn} = 0.075W^{0.75} [(1 + 0.90 (gn)]$$

where NE_{m+gn} is in megacalories per day and W is in kilograms per day and daily weight gain (gn) is in kilograms per day. If gn (gain) = 1 kilogram then $NE_{m+gn} = 0.136W^{0.075}$ for steers and $0.143W^{0.075}$ for heifers and by subtracting the maintenance requirement ($0.075W^{0.075}$) the NE_{gn} requirement for one kilogram of gain can be expressed as $0.0161W^{0.75}$ for steers and $0.068W^{0.75}$ for heifers. The NE_m and NE_{gn} requirements presented in table 32 were calculated using these expressions.

To make the best use of the data on NE_m and NE_{gn} requirements it is necessary to have comparable values on feeds. By use of the "difference trial" technique these values can be determined (Lofgreen, 1964 and 1965). The NE_m and NE_{gn} values

Table 31. Determination of the values of k for the net energy requirements for weight gain

Ration	Replication	Number of animals	Mean $W^{0.75}$	Daily NE_m (a)	Daily energy retained	Daily weight gain (gn)	'k'
			kg	megcal/$W^{0.75}$	megcal	kg	
Steers							
Basal + 60% grain restricted	1	6	74.2	0.075	2.65	0.64	0.74
	2	6	69.5	0.075	2.10	0.55	0.73
	3	6	71.4	0.075	2.82	0.62	0.85
Basal + 70% grain ad libitum	1	6	78.4	0.075	5.75	1.15	0.85
	2	6	78.0	0.075	5.33	1.09	0.84
	3	6	78.3	0.075	5.71	1.12	0.87
						Steer mean	0.81
Heifers							
Basal + 60% grain restricted	1	6	65.1	0.075	2.75	0.61	0.92
	2	6	66.6	0.075	2.67	0.58	0.92
	3	6	66.5	0.075	2.88	0.63	0.92
Basal + 70% grain ad libitum	1	6	72.2	0.075	5.18	1.07	0.89
	2	6	72.9	0.075	5.21	1.02	0.93
	3	6	72.1	0.075	5.36	1.10	0.90
						Heifer mean	0.91

for alfalfa hay containing 28 percent crude fiber were found to be approximately 1.19 and 0.53 megacalories per kilogram, respectively. Corresponding values for barley weighing approximately 630 grams per liter were 1.87 and 1.10 megacalories per kilogram on an air dry basis. To satisfy the maintenance requirement of a 300-kilogram steer it would require 4.55 kilograms of alfalfa hay (5.41/1.19). For this animal to gain 0.5 kilogram per day it is necessary to supply, in addition to the maintenance requirement, 2.20 megacalories of NE_{gn} (0.5 x 4.40). It would require 4.15 kilograms of alfalfa hay to furnish the 2.20 megacalories of NE_{gn} (2.20/0.53) making a total of 8.70 kilograms of alfalfa to meet the maintenance and gain requirements of a 300-kilogram steer. A 300-kilogram heifer would require 9.17 kilograms of hay to make the same gain.

One of the criticisms of the NE system has been its failure to give roughage feeds their proper value for maintenance and it is common to recommend the use of DE values in comparing feeds

Table 32. Net energy requirements of growing-finishing cattle

Body weight	For maintenance (NE_m)	For production per kg of gain (NE_{gn})		Body weight	For maintenance (NE_m)	For production per kg of gain (NE_{gn})	
		Steers	Heifers			Steers	Heifers
kg	megcal per day			kg	megcal per day		
150	3.22	2.62	2.92	350	6.08	4.94	5.51
160	3.38	2.75	3.06	360	6.20	5.04	5.62
170	3.53	2.87	3.20	370	6.33	5.15	5.74
180	3.69	3.00	3.35	380	6.46	5.25	5.85
190	3.83	3.12	3.48	390	6.59	5.36	5.97
200	3.99	3.25	3.62	400	6.71	5.46	6.09
210	4.14	3.37	3.75	410	6.83	5.56	6.19
220	4.28	3.48	3.88	420	6.96	5.66	6.31
230	4.43	3.61	4.02	430	7.08	5.76	6.42
240	4.58	3.72	4.15	440	7.21	5.86	6.53
250	4.72	3.84	4.28	450	7.33	5.76	6.64
260	4.68	3.95	4.41	460	7.45	6.06	6.75
270	5.00	4.06	4.53	470	7.57	6.15	6.86
280	5.14	4.18	4.66	480	7.69	6.25	6.97
290	5.27	4.29	4.78	490	7.81	6.35	7.08
300	5.41	4.40	4.90	500	7.93	6.45	7.19
310	5.54	4.51	5.03	510	8.05	6.55	7.30
320	5.68	4.62	5.15	520	8.17	6.64	7.41
330	5.81	4.73	5.27	530	8.28	6.73	7.51
340	5.94	4.83	5.39	540	8.40	6.83	7.62

for maintenance. For example, alfalfa hay and barley contain approximately 2.31 and 3.48 kilocalories, of DE per kilogram (NRC, 1963). On this basis alfalfa hay is 66 percent as valuable as barley grain. The estimated NE values for the two feeds listed by Morrison (1956) are approximately 0.88 and 1.57 megacalories per kilogram. On this basis alfalfa hay is only 56 percent as valuable as barley. It is commonly claimed that the comparison made on the basis of the DE values is a more accurate estimate of the relative value of roughages for maintenance, but the NE values are more accurate for production. It is interesting that the proposed system of NE evaluation based upon NE_m and NE_{gn} gives roughages a relatively higher value for maintenance than for production. For example, on a basis of NE_m alfalfa hay is 64 percent as valuable as barley grain but only 48 percent as valuable for production (gain). It seems, therefore, that the system based on NE_m and NE_{gn} values overcomes this common criticism of the NE system of feed evaluation.

Table 33 lists the NE_m and NE_{gn} values for some common feeds.

Table 33. Net energy content of some feeds

Feed	Dry matter	As fed		Dry	
		NE_m	NE_{gn}	NE_m	NE_{gn}
	%	megcal/kg		megcal/kg	
Alfalfa, hay, 28% fiber	89.7	1.19	0.53	1.33	0.59
Animal, fat	98.0+	4.53	2.59	4.58	2.62
Barley, straw	88.2	0.70	0.31	0.79	0.35
Cottonseed, hulls	90.3	0.95	0.42	1.05	0.46
Corn, aerial part, ensiled+	29.0	0.53	0.24	1.83	0.83
Barley, grain	89.0	1.87	1.10	2.10	1.24
Beet, pulp with molasses, dehydrated	90.9	1.83	1.08	2.01	1.19
Cottonseed meal, solvent extracted+	92.0	1.72	1.01	1.87	1.10
Sorghum, milo, grain Sacramento Valley	88.0	1.87	1.10	2.12	1.25
Sugarcane, molasses, to 15% of ration	75.0	1.37	0.78	1.83	1.04
Wheat, mill run	90.0	1.75	0.91	1.94	1.01

+ Estimated values.

Supplementation as a Tool in Studies of Nutrient Requirements

Wyoming research on nutrient requirements has concentrated on testing the National Research Council requirements for sheep (1957) although a revision of the requirements has been made (1964) since their research was initiated. Their research has been concerned with the requirements for growing replacement ewe lambs. The technique involved the feeding of rations designed to provide 60, 80 and 100 percent of the NRC protein and energy requirements and 75 and 100 percent of the phosphorus requirements. The following results have been found to date:

1. The 80 percent protein level appeared to be adequate for wool growth since the wool from ewes fed the 80 or 100 percent protein levels had significantly longer staple and larger diameter than the wool from ewes fed the 60-percent protein level (Hashem *et al.*, 1964).

2. The protein levels in the ewes' ration did not significantly influence wool shrinkage or the development of primary and secondary follicles in their lambs during the prenatal stage of life (Hashem *et al.*, 1964).

3. Energy and phosphorus levels in the ewes' ration did not significantly influence staple length, fiber diameter, wool shrinkage, or the development of primary and secondary follicles in their lambs during the prenatal stage of life (Hashem *et al.*, 1964).

4. The levels of the protein, energy and phosphorus in the ewes' diet did not influence the postnatal development of follicles in their lambs (Hashem *et al.*, 1964).

5. The weight gains followed the level of protein and energy in the diet with the 80 and 60 percent levels producing suboptimum gains. Phosphorus level, however, did not significantly influence weight gains (Kercher and Gallinger, 1963).

6. When mature ewes were fed rations adequate in protein and phosphorus with 100 percent of the NRC energy requirement, the ewes gained approximately twice as much weight during gestation, gave birth to slightly heavier and more vigorous lambs, lost less weight after parturition, and their lambs gained about the same amount of weight up to 56 days of age as ewes fed rations adequate in protein and phosphorus but limited to 80 percent of the NRC energy requirement.

The Colorado, Idaho, Montana, Nevada, and Oregon Stations have studied nutrient requirements of grazing livestock by measuring animal response to various levels of nutrient supplementation.

Colorado workers supplemented cows being wintered on native sandhill ranges with three levels of dehydrated alfalfa and measured their performance. Table 34 presents the results of this study.

In a summer supplementation test, the Colorado workers found that maintaining a crude protein percentage of 6.5 percent in the diet produced calves weighing approximately 9 kilograms more at weaning than those receiving no supplement.

In a study to determine the influence of protein supplementation on range forage digestibility, the Idaho Station used rumen fistulated heifers in which digestibility was determined by the nylon bag method (Howard, 1963). The heifers were maintained together in a partially covered corral throughout the study and grass hay was fed *ad libitum*. In addition to the hay, the experimental rations consisted of approximately 0.9 kilogram per head per day of (1) no supplement; (2) 20 percent crude protein pellets; (3) 32 percent crude protein pellets; and (4) 41 percent crude protein cottonseed meal pellets. The supplements were fed individually once daily to the heifers in stanchions.

Triplicate, 10-gram samples of four cellulose sources were suspended in nylon bags in the rumen during each of the 24-hour periods. The cellulose sources were cotton gauze; the grass hay being fed, green crested wheatgrass, and dry crested wheatgrass. All samples were oven dried at 55 degrees C. and weighed prior to being placed in the rumen. Upon removal from the rumen all samples were carefully washed, oven dried and reweighed. The samples were washed by repeated swirling in water until the rinsings were

Table 34. Effect of winter supplementation on cows grazing native sandhill range

Item	Level of supplement		
	Light	Medium	Heavy
Amount of daily supplement, kg	0.23	0.68	1.14
Percent loss in weight from November to May	15.9	14.4	10.8
Conception %	98.9	95.6	94.4
Conception on first service, %	71.9	77.5	77.3
Birth weight, kg			
Males	33.1	34.4	35.2
Females	31.7	32.1	32.6

clear. Care was exercised to avoid squeezing or kneading the bags. The dry matter weight loss was calculated from the difference between the initial and final weights.

The average percentage dry matter losses from the nylon bag samples are shown by protein supplement and cellulose source in table 35. The mean square for cellulose source, as expected, was significant ($P < 0.01$) and each of the sources was different from the others as measured by Duncan's multiple range test. Apparently protein supplementation did not stimulate cellulose digestibility in this trial except that there was a tendency for the 20 percent and the 32 percent levels to reduce digestibility. No reason for this is known.

The effect of varying the phosphorus levels in the supplement fed to bred yearling heifers on phosphorus-deficient winter range has been studied in Montana (Thomas *et al.*, 1965). A comparison of 10 and 20 percent protein levels in the supplement was also made. The 3-year average weaning weights of calves from unsupplemented cows and those fed approximately 0.9 kilogram of supplement containing 0.5, 1.0, 1.5 and 2.0 percent phosphorus were 183, 202, 207, 201 and 205 kilograms, respectively. All levels of phosphorus significantly increased the weaning weight over the unsupplemented group with no significant differences occurring among phosphorus levels. It is not known how much of the response was caused by the extra feed and how much was caused by the phosphorus. Increasing the protein in the supplement from 10 to 20 percent increased weaning weight from 210 to 216 kilograms, respectively, a significant increase.

On a 95-percent browse type range in southern Nevada, a winter protein supplement fed to weanling calves brought about

Table 35. Effect of protein level upon digestibility (percent dry matter loss)[+]

Cellulose source	Percent protein in pellets				
	0	20	32	41	Means §
Cotton gauze	15.57	13.37	14.16	15.83	14.73^{a}
Grass hay	53.40	50.24	50.25	52.36	51.56^{b}
Green crested wheatgrass	59.62	56.94	57.12	59.70	58.34^{c}
Dry crested wheatgrass	44.41	41.72	41.94	44.71	43.20^{d}
Means	43.25^{a}	40.56^{c}	40.87^{bc}	43.15^{ab}	

[+] Each value in the table is the mean of 24 determinations.

§ Mean values in the same protein level or cellulose source having common superscripts (a, b, c, d) are not significantly different.

a significantly greater gain over the unsupplemented group than did an energy supplement (Bohman, *et al.*, 1961). When the gains for the entire year were compared, a winter supplement of 0.45 kilogram of barley per head daily failed to significantly affect gain. However, a winter supplement of 0.45 kilogram per head daily of either cottonseed meal or soybean meal or 1.36 kilograms of alfalfa significantly increased the gain over the unsupplemented or barley supplemented group. A supplement of 8 grams per head daily of phosphorus for the entire year caused a growth stimulation equal to that of winter protein supplementation. These studies also showed that a winter protein supplement was more effective than an energy supplement in maintaining hemoglobin and hematocrit levels. A similar study with cows (Speth, *et al.*, 1962) showed that cows receiving either protein or energy supplements maintained body weight significantly better than unsupplemented cows. Those receiving protein from cottonseed meal or soybean meal had significantly more calf deaths at birth than the unsupplemented group or those supplemented with protein from alfalfa or energy from barley. The same blood effects caused by protein supplements were observed in the cows as in the calves. In a subsequent study (Speth *et al.*, 1963) weanling calves were fed a protein supplement alone at two levels and in combination with an energy supplement during the winter supplemental feeding period. A summary of the effects on weight gains is shown in table 36.

The nutrient supplementation work at the Oregon Station involved estimation of the nutrient intake from the forage grazed and provided sufficient supplemental nutrients calculated to produce a specified level of performance. For example, observations on a specific range indicated that cattle early in the grazing season would gain approximately 1.02 kilograms per day with no supple-

Table 36. Effect of supplements on the gain of weanling calves on semi-desert range

Number, weight, or seasonal gains	Cottonseed meal, kg			
	0	0.45	0.9	0.45
		Barley, kg		
	0	0	0	0.45
Number of calves	20	20	20	20
Initial weight, kg	149	151	148	151
Winter daily gain, 158 days, kg	0[a]	0.14[b]	0.18[bc]	0.21[c]
Summer daily gain, 188 days, kg	0.36	0.36	0.31	0.31
Yearly daily gain, 346 days, kg	0.18[a]	0.25[b]	0.25[b]	0.26[b]

[abc] Within the same category, means having different superscripts are significantly different ($P < .05$).

ment. Later in the season, however, both a protein and energy supplement were needed. Based upon predicted nutrient consumption from the range forage the amount of supplement necessary to gain 1.02 kilograms was calculated. Treatment 1 in table 37 represents control cattle with no supplement while treatment 2 cattle received the supplement calculated to support a daily gain of 1.02 kilograms. Treatment 3 received extra energy supplement early in the grazing season. During the first 56 days of the grazing season the unsupplemented cattle gained at the desired level and the protein supplement apparently was not needed although the extra energy furnished treatment 3 stimulated gains. During the last 28 days, however, the gains of the unsupplemented cattle dropped markedly while those receiving the required level of supplement remained near the desired level. The overall performance of the cattle receiving the calculated requirement was in close agreement with the predicted performance. Those receiving extra energy during the early period gained slightly more. The technique of estimating nutrient consumption and supplementing for a specific performance appears to hold promise provided the estimate of forage consumption is good and the nutrient requirements for the desired level of performance are valid. The Oregon work has indicated that higher rates of supplementation may reduce range forage consumption to such an extent that the desired performance is not achieved.

Table 37. Response of cattle to calculated level of supplementation

Item	Treatment 1	2	3
Period 1 (May 10 — June 7):			
Barley supplement, kg per day	0	0	0.31
Cottonseed meal supplement, kg per day	0	0	0
Mean daily gain, kg	1.08	1.12	1.27
Period 2 (June 7 — July 5):			
Barley supplement, kg per day	0	0.04	0.07
Cottonseed meal supplement, kg per day	0	0.28	0.28
Mean daily gain, kg	1.02	0.96	1.29
Period 3 (July 5 — August 2):			
Barley supplement, kg per day	0	0.27	0.27
Cottonseed meal supplement, kg per day	0	0.48	0.48
Mean daily gain, kg	0.67	1.10	0.81
Entire period (May 10 — August 2):			
Total barley supplement, kg	0	8.68	18.20
Total cottonseed meal supplement, kg	0	21.28	21.28
Mean daily gain, kg	0.92	1.06	1.12

EXPERIMENTALLY FEEDING RANGE LIVESTOCK

Summary

Cattle may be experimentally supplemented under range conditions with either group or individual feeding. Special care is required with group feeding to avoid confounding experimental effects with environmental conditions. Individual feeding of supplements usually decreases or eliminates the above biases but is more laborious. Various methods are described for feeding experimental animals under range conditions. Range animals need not be supplemented daily. If watering schedules developed by the animals are correlated to feed supplementing schedules, the labor involved in feeding animals is greatly reduced. A detailed plan is presented to minimize variations and to avoid management biases in weighing experimental animals under range conditions.

Introduction

In addition to studies on the botanical and nutritive composition of an animal's diet and the factors that affect the chemical composition of a given range species, supplemental feeding trials, both detailed and practical, using some of the most valuable supplements in various combinations and amounts and during the different seasons must be conducted.

In planning experiments to determine which supplements to feed, several levels and ratios of nutrients should be fed above and below the normal level in order that the correct economic recommendation can be given to a rancher (figure 6). If the supplement is inexpensive it should be fed near the maximum level, if expensive, near the minimum. Too often, experiments are conducted only over a very limited range, without obtaining information on each side of this point.

In planning supplementary feeding trials under range conditions, the measurement of variation without bias is a difficult problem. Sources of variation include those due to treatment and uncontrolled variables. It is essential that uncontrolled variations be minimized or measured in order that treatment differences can be assessed. Uncontrolled variations consist of differences among animals and differences among pastures. On range pastures these differences are particularly large because of variations in vegetation, topography, water, soil and climate.

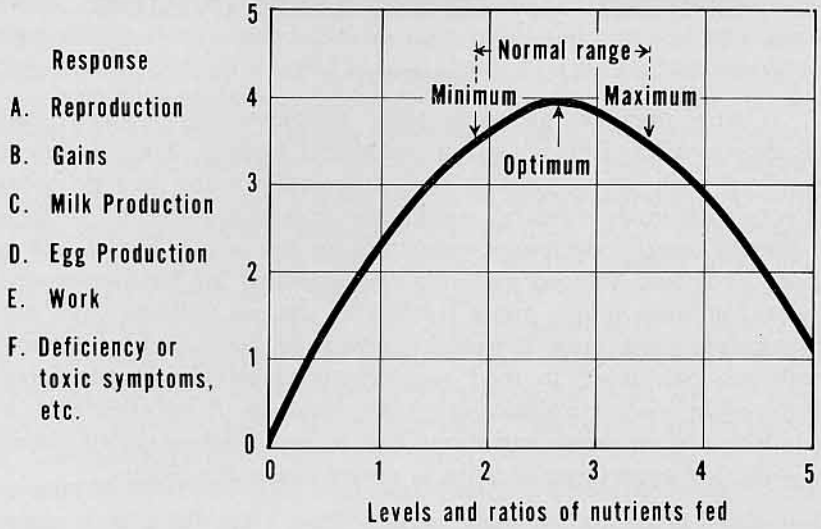


Figure 6. Response curve of animals fed various levels and ratios of nutrients. Levels and ratios of nutrients should be fed below and above the normal range to determine the economic level of feeding. (Courtesy Lorin E. Harris, Utah Agricultural Experiment Station, Logan).

Several ways have been devised to feed supplements experimentally on the range. Usually groups of livestock are enclosed in a series of pastures with each group receiving a different treatment. However, it is difficult to find pastures that are uniform enough in plant composition and topography so the results are not influenced by pasture variability. This is true even though the animals are rotated among pastures.

Group Feeding

Cattle

Detailed studies can best be conducted by feeding trials in several localities to ascertain the kinds and amounts of supplements that should be fed to correct dietary deficiencies during various seasons. On western winter ranges 10 to 80 acres of grazing area is required per cow. Therefore, there is a high cost for fencing pastures for the number of cattle necessary to effectively measure pasture differences. This has limited the progress of range cattle research.

The Utah Station has used the following method to assess pasture variation. Treatments are allotted at random to a block

of pastures. Within each block the supplementary treatments are rotated among the pastures in order that each supplement is fed in every pasture. Preferably four or more blocks of pastures should be used. Too often only one block of pastures is used and treatment and pasture effects are confounded.

Sheep

With group feeding, the number of animals should be larger than for individual trials; however, to assess the variability more than one group should be fed the same treatment unless there are many hidden replications in a factorial design. The usual design of having one small group of animals per treatment does not give the necessary information to assess variation. A better plan is to have four or more groups per treatment.

All animals may graze one area in group feeding on the range. At time of supplementing they are divided by a 4-way cutting chute (figure 7). Animals can be further separated into 9 groups by sorting twice. If supplements are fed every other day to two replicated groups of animals, 18 groups may be separated and fed at one location. The number of groups could also be increased by having all treatments represented in several groups of animals on several areas having more than one four-way cutting chute. Montana and Utah research workers have used such cutting chutes (Van Horn *et al.*, 1952; Clanton *et al.*, 1956; Harris *et al.*, 1959).

Individual Feeding

Most of the disadvantages of group feeding may be overcome by feeding animals individually. Under this system all animals are grazed on the same pasture, brought to one location and fed supplements every second day in individual pens. Portable pens permit moving of the feeding facilities to various locations. Under this system all animals have access to the same basal diet of range forage. Animals can also be weighed at random under standard conditions. Variations may be assessed without bias and there is no confounding of pasture and treatment effects. For additional precision and replication, the treatments can be repeated in different locations with several sets of portable pens. Fencing of the range is not necessary and animals can be controlled by herders or by controlling access to water.

Portable feeding pens, corrals and weighing crates for feeding

Figure 7. A corral and four-way cutting chute for sheep. The corral includes two holding pens and four pens where the animals can be fed. By running the sheep through the chute twice, nine groups can be obtained. (Courtesy Lorin E. Harris, Utah Agricultural Experiment Station, Logan).

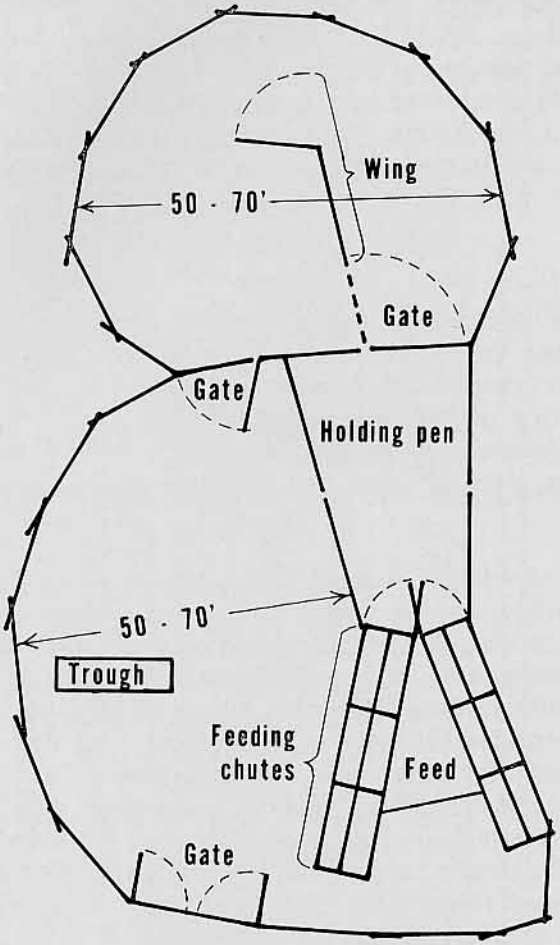
and weighing range sheep individually have been designed and utilized (Harris *et al.*, 1952). Through the use of this equipment it has been possible to do research in many areas and carry on experiments which take into account the variability of vegetation and climatic conditions. The same general plan has been used to design feeding and weighing equipment to carry on similar work with range cattle (Harris *et al.*, 1967; Bohman *et al.*, 1961).

Cattle

Cattle used in experiments should be selected at random from average range herds. These animals normally graze both summer and winter ranges and usually have been handled very little prior to being placed on experiment. They may be identified by means of bronze number tags attached to small chains around their necks, large ear tags, hair dyes, freeze branding, or similar means.

Cows should be assigned to treatments at random. Supplemental feeding may be made at one, two or three-day intervals and given to animals at random times. Cows are allowed to graze at distances as great as 6 miles in any direction from the feed corrals. Daily feeding of supplements is usually unsuccessful, as it takes considerable time to gather and feed the cattle. This leaves insufficient time for the cattle to graze. Cattle in an area will come to water rather regularly every other day. When feedings and waterings are correlated little time is needed to put the cattle in the chutes for feeding. The Utah and Nevada investigators have used this system. The corral layout and individual pens are shown in figures 8 and 9.

Water may be critical. It is preferable to have the water near or in the corrals. When cattle come for a drink they may be segregated and fed their supplements. A shortage of water or dirty water will cause cattle to leave the area in a short time. Ten days time may be required to establish a feeding pattern, but after this even wild cows may usually be fed in the chutes without difficulty. It requires about 2 hours maximum time to feed 60 head of cattle. If the feedings are not correlated with the water, Montana studies have found that cattle may be fed three times a week. The weeks feed may be fed on a pro-rated basis on Monday, Wednesday and Friday.



Materials required

12	12 in. x 8 in. x 4 in. feed boxes
10	12 ft. panels 5ft. high
22	8 ft. panels 5 ft. high
10	4 in. panels 5 ft. high
5	55 ft. rolls of 5 ft. high net wire
20 to 30	18 ft. pine poles
12	24 in. chains with snap fasteners
60 - 70	6 ft. steel posts
Smooth wire to tie fence parts together	

Figure 8. Diagram of corral and inventory of materials for feeding cattle individually. (Courtesy John E. Butcher and Lorin E. Harris, Utah Agricultural Experiment Station, Logan).

Sheep

For individual feeding experiments with sheep, the animals are herded on the open range every day, are corraled at night, and may be fed every other day in individual portable pens (figure 10). The control sheep are marked with a black number, while the sheep fed supplements are marked with a red number. After about 2 weeks the red-numbered sheep readily go into the pens where they are fed the supplements. The supplements are weighed and placed in small paper bags in a tray previous to feeding. No attempt should be made to put a certain animal in a certain pen. Rather, the number on the sheep is read, and the similarly-numbered bag is selected from the feed tray and delivered to the animal. Using this procedure and 56 individual pens, it is possible to feed 156 sheep in about 2 hours.

If snow is not available during the winter, the sheep should be watered every second day from a water tank on a truck. Portable troughs should be moved every time the sheep are watered to prevent trampling of the range. To check whether or not individual feeding, weighing, and handling of the sheep depresses performance, sheep of identical ages should be selected at random

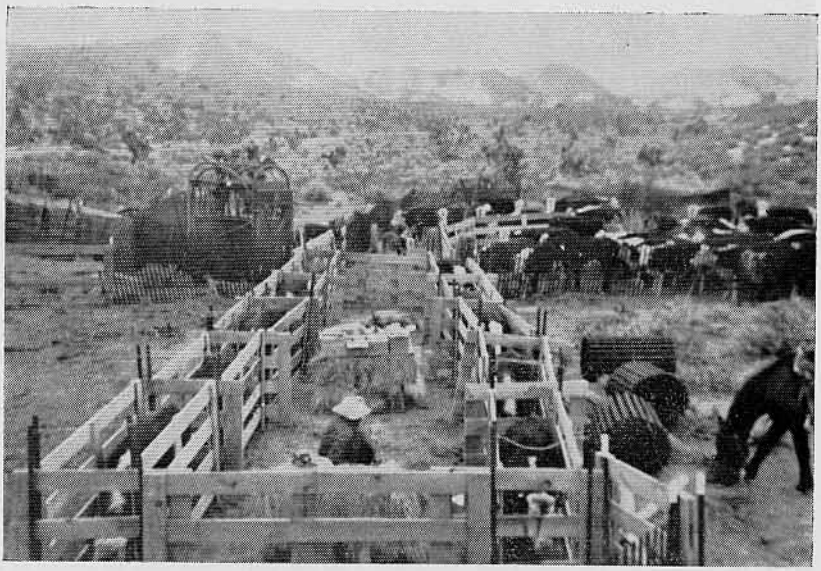


Figure 9. Corrals and chutes used to feed cattle in individual feeding trial. (Courtesy V. R. Bohman, University of Nevada).

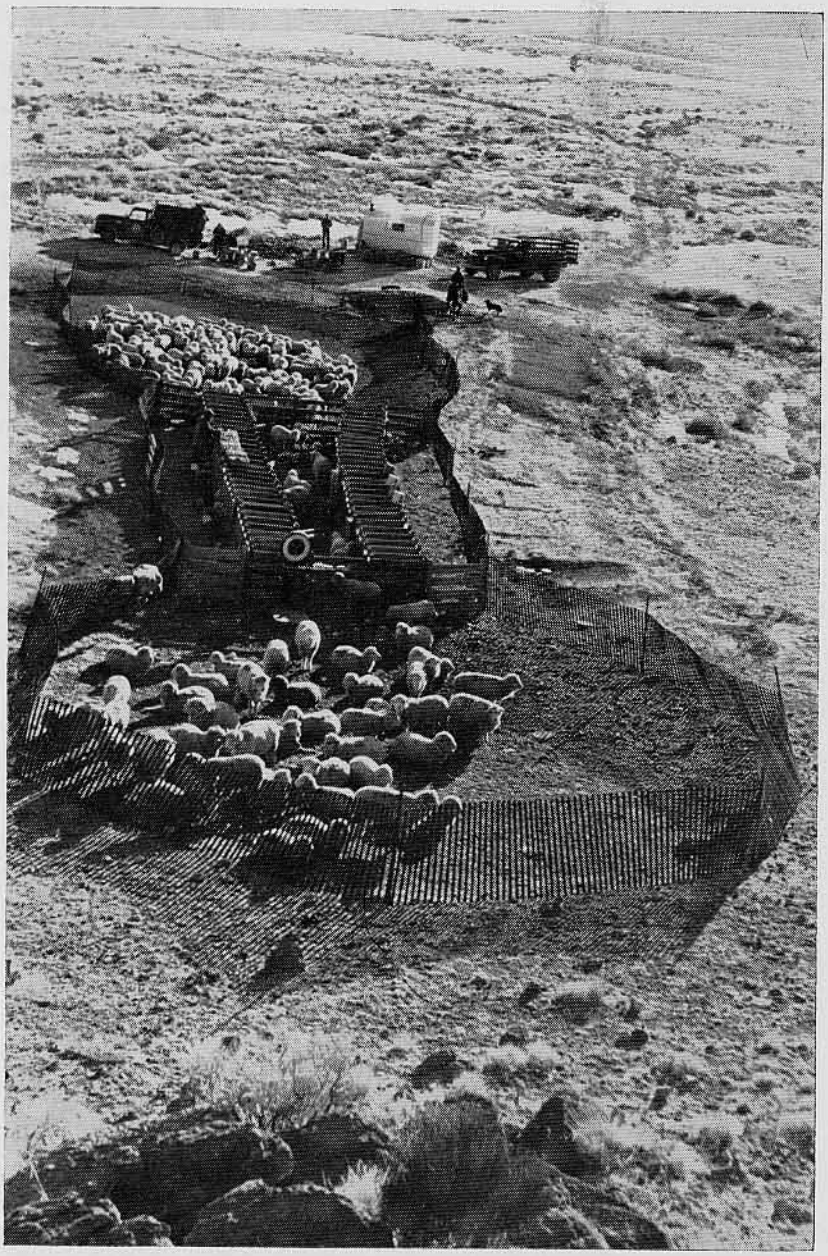


Figure 10. Individual portable pens for sheep. Holding corrals are on each end. Pens are moved every 2 to 3 weeks. Trays are used to hold each sheep's feed. Sheep are fed in buckets. (Courtesy Lorin E. Harris, Utah Agricultural Experiment Station, Logan).

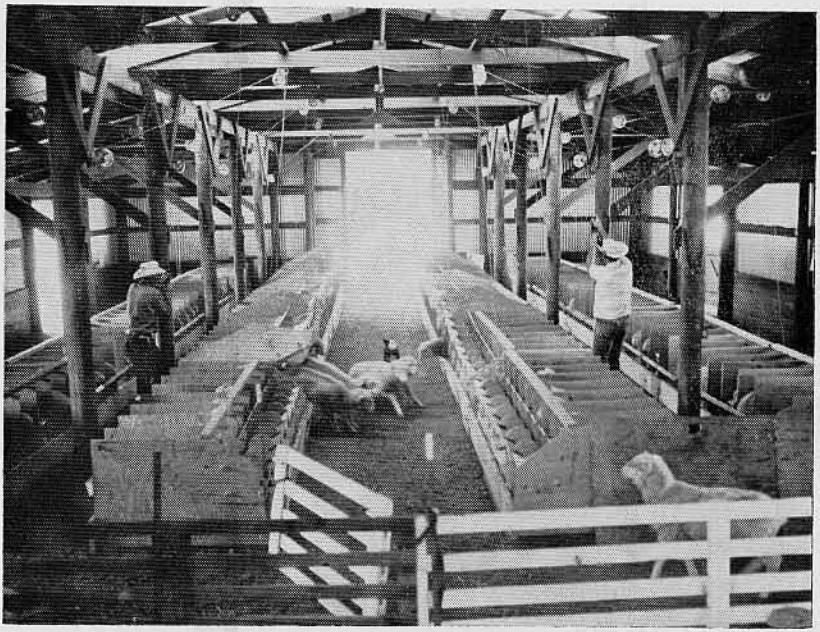


Figure 11. Individual pens for lambs and yearlings which are in use at the Hopland Station. (Courtesy of D. T. Torell, California Experiment Station, Hopland).

from the experimental herd, weighed and placed in some large herd. The result of this comparison for several consecutive years usually shows that there are no statistically significant differences between the two methods of handling the sheep (Harris *et al.*, 1952).

The California Station has devised individual pens for lambs and yearlings which are being tested at the Hopland Field Station (figure 11).

Weighing Animals

Methods of weighing animals on experiments have been reviewed by Baker *et al.* (1947), Bean (1948), Lush *et al.* (1928), Patterson (1947), and Whiteman *et al.* (1954).

Care should be taken to avoid bias between treatments; all animals should have the same intestinal fill within a replication or within a block of treatments when weighed. The average variation from day to day was least and the range between extremes

was narrowest immediately before feeding in the morning (Allen, 1946).

With these facts in mind, it is suggested that weighing procedures be standardized as follows:

1. Gather animals late at night or at daylight and put them in a corral without feed or water. Begin weighing soon after daylight, but give the animals sufficient time to urinate and defecate before weighing is started. Follow the same procedure each time.

2. Mix all animals in a large corral within a replication or block of treatments and weigh at random. Avoid weighing animals by lot, pasture, or treatments as this creates a bias among treatments.

3. One weighing is sufficient if there are a number of animals per treatment. If accuracy is wanted on individual animals, weights should be taken on 2 or 3 different days (Koch *et al.*, 1958).

4. If absolute gains in weight are needed, a standardizing period with the same kind of feed at the beginning and end of the trial is desirable to reduce variability in intestinal fill (Balch and Line, 1957).

Sheep are often weighed every 28 days with a portable platform scale and weighing crate. Normally, they will jump into the weighing crate with little assistance if the front door is raised simultaneously with the back door.

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