AN ABSTRACT OF THE THESIS OF

Michael Lindsay McInnis   for the degree of ___Master of Science___

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Title: A COMPARISON OF FOUR METHODS USED IN DETERMINING

THE DIETS OF LARGE HERBIVORES _____

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⁀William C. Krueger

Esophageal fistulation, stomach content analysis, fecal analysis, and ocular-estimate-by-plot are four of the most publicized methods of determining the diets of large herbivores. The principal objective of this study was to compare the relative values of each of these methods based upon information collected from bi-fistulated (esophageal and rumen) sheep selecting their own forage from a tall-forb community in early summer and late summer, and from confined sheep fed a hand-mixed diet of known composition.

Microscope slide mounts were made of plant fragments collected from the esophagus, rumen, and feces of sheep grazing a beauty cinquefoil/velvet lupine - Kentucky bluegrass/timothy community in northeastern Oregon. Ocular estimates of forage utilization were made concurrently. Data were converted to percent composition on a dry weight basis for comparisons. Significant differences (p < .05) in percent diet composition among methods occurred for 18 of the 31 plant species consumed in the early summer

trial, and for 17 of the 31 plant species consumed in the late summer trial.

Diets as determined by the ocular-estimate-by-plot method were generally lower in graminoids and higher in forbs than those as determined by other methods. The esophageal fistula method yielded diets that were lower in graminoids and higher in forbs than diets as determined by stomach content analysis and fecal analysis. Diets as determined by fecal analysis were higher in graminoids and lower in forbs than diets as determined by other methods.

In the second study phase, ten confined bi-fistulated sheep were separately fed a dry, ground, hand-composited mixture of orchardgrass, fawn fescue, alfalfa, and ladino clover. The botanical composition of this mixture was determined by microscopic examination of plant fragments and compared to the botanical composition of diets as determined by microscopic examination of esophageal, rumen, and fecal samples.

The composition of each of the species contained in esophageal samples was similar to that of the actual diet. Rumen samples were similar to the actual diet only in their composition of orchardgrass, and contained significantly higher (p < .05) amounts of total grasses and significantly lower (p < .05) amounts of total forbs than the actual diet and esophageal samples. Fecal samples were significantly higher (p < .05) in their composition of grasses and significantly lower

(p < .05) in their composition of forbs than the actual diet and

samples collected from the esophagus or rumen.

A Comparison of Four Methods Used in Determining
the Diets of Large Herbivores

by

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in charge of major

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Associate Professor of Animal Science
in charge of thesis

Redacted for Privacy

Leader, Rangeland Resources Program

Redacted for Privacy

Dean of Graduate School

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A COMPARISON OF FOUR METHODS USED IN DETERMINING THE DIETS OF LARGE HERBIVORES

INTRODUCTION

An awareness of the diets of herbivores is an important factor in developing management programs. Such knowledge is essential to determine how well native and seeded forages meet the nutritional requirements of grazing animals. Knowing the foods of animals puts the manager in a position to alter plant composition on the range to influence or control animal populations. Where several types or classes of animals are using the same range, knowledge of their respective diets allows the manager to adjust numbers to keep pace with the food supply.

Microscopic examination of plants recovered in fragmentary condition from esophageal and rumen fistulae, and fecal material are three of the most publicized methods of determining the food habits of large herbivores. A fourth approach to estimating consumption is by observing or measuring utilization of forage plants. Associated with each of these methods are a number of advantages and disadvantages which have stimulated discussion as to which is most useful in interpreting food habits of large herbivores.

To this point, a comparison of the relative values of these methods has not been made with animals on the same diet. Partial

studies have indicated that various methods yield significantly different results.

The objectives of this research were as follows:

1. Determine the diets of sheep (Ovis aries) grazing the same range in early summer (early July) and late summer (late August) by the esophageal fistula, stomach content analysis, fecal analysis, and ocular-estimate-by-plot methods.

2. Compare the diet compositions among methods.

3. Determine the diets of sheep fed a hand-composited diet of known composition using the esophageal fistula, stomach content analysis, and fecal analysis methods.

4. Determine the accuracy and relative values of the esophageal fistula, stomach content analysis, and fecal analysis methods by comparing the composition of the diet as estimated by each method to that as determined by each of the other two methods, and to the known composition of the hand-composited diet.

DESCRIPTION OF STUDY AREA

This investigation was undertaken on the Eastern Oregon Agricultural Research Center at Union, Oregon. Two different locations were used as experimental areas. Rangeland grazing trials were conducted on the Hall Ranch unit of the Research Center while feeding trials were carried out on the Research Center proper.

The Hall Ranch is located in the lower Catherine Creek basin of Union County in the foothills of the Wallowa Mountains. The ranch lies 19 km southeast of Union in Township 5 South, Range 41 East of the Willamette Meridian. The specific site chosen as the experimental area was located in the southwest quarter of the ranch (Figure 1).

Geology and Physiography

The geologic history of the Wallowa Mountains has been described by several authors (Lindgren, 1901; Goodspeed, 1939; Smith and Allen, 1941; Krauskopf, 1943; Wagner, 1955; Hampton and Brown, 1963). A summary of geologic events is presented here to enhance understanding of the ecosystem in which this study was conducted.

The Wallowa Batholith was originally a dome-shaped mass of granite approximately 32 km in diameter (Lindgren, 1901). During the Miocene Epoch, Columbia River basalts covered this entire area

HALL RANCH

STUDY AREA

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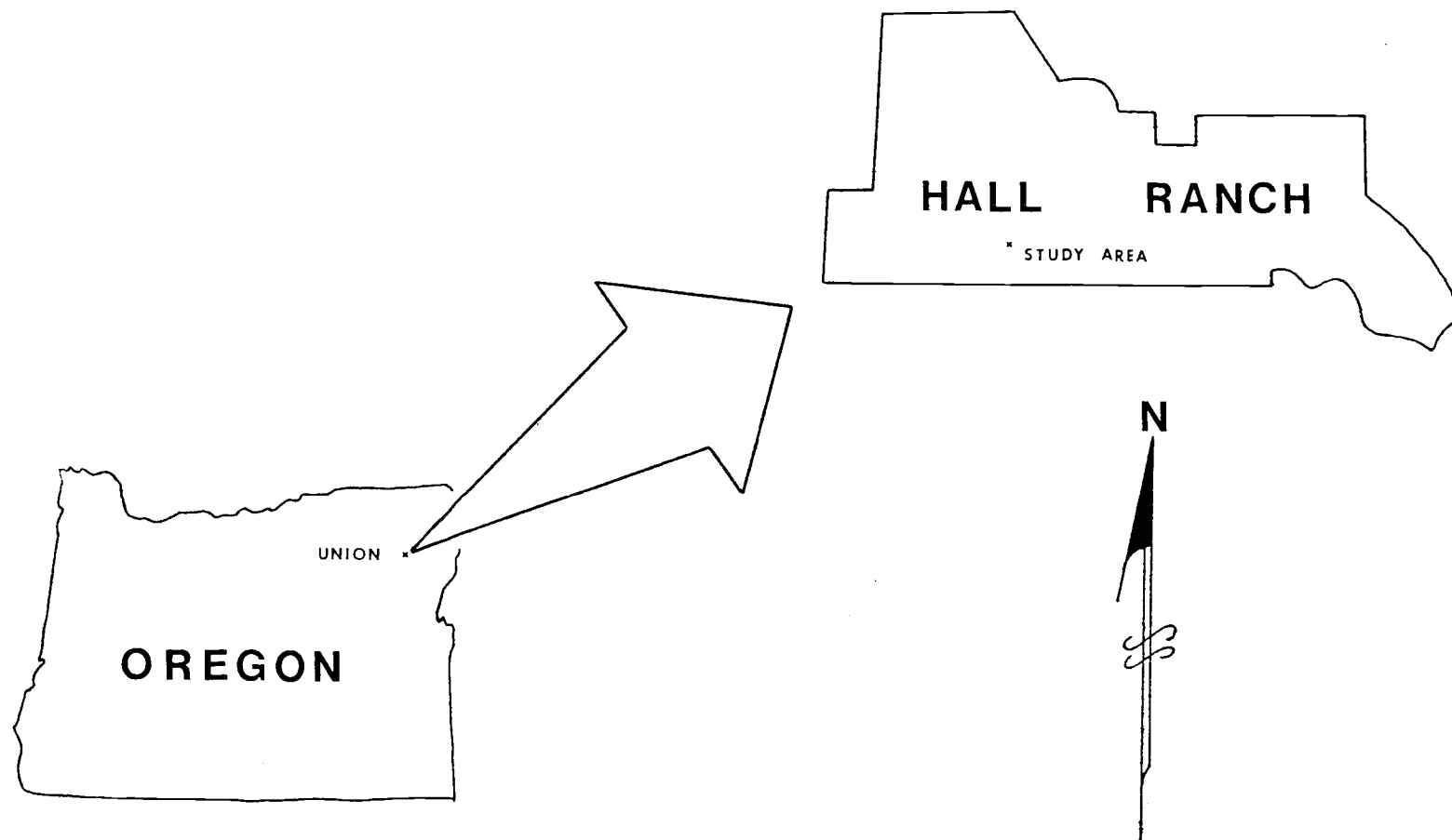
UNION

OREGON

Figure 1. Location of the study area on the Hall Ranch of the Eastern Oregon Agricultural
Research Center near Union, Oregon.

to a depth of several thousand meters (Smith and Allen, 1941).
Diastrophic processes during the late Tertiary-Quaternary lifted the
Wallowa Mountains to their present heights (Wagner, 1955) creating
a fault block some 80 km long by 32 km wide (Root et al., 1960).
Subsequent erosion resulted in the steep ridges, broad valleys and
alluvial plains now common in the area.

Catherine Creek, which ultimately flows into the Columbia
River by way of the Grande Ronde and Snake Rivers, bisects the
Hall Ranch. The eastern segment of the ranch is underlain by lava
flows tilted to the southwest (Pettit, 1968). The western portion lies
on a 914 m fault escarpment. Elevations of the Hall Ranch range from
1036 m to 1249 m. The study area is situated on a 15% north facing
slope with an eastern aspect and an elevation of 1128 m.

Climate

The Hall Ranch receives the bulk of its precipitation as snow
and rain during the cold winter months of November through
February. Fall and spring are cool and moist while summers are
hot and dry. Maximum temperatures rarely exceed $38^{o}C$ though
freezing or near freezing temperatures are common every month.
The average annual precipitation on the Hall Ranch is 63.5 cm.

A weather station located nearly 1 km from the study area has
yielded monthly temperature and precipitation data since 1964. The

mean annual precipitation at this weather station for the past ten

years is 58.4 cm. Precipitation and temperature data for the past

ten years are summarized and compared with 1975 data in Figure 2.

Tabulated weather data for this station are found in Appendix A.

Soils

The soils on the study area belong to the Wilkens series

(Experiment Station file data, 1964). This soil series is described in

detail in the Soil Survey of the Starkey Experimental Forest and Range

(U.S.D.A., 1960), and also discussed by Strickler (1965).

The Wilkens series is comprised of imperfectly drained, fine-

textured "Alpine Meadow" soils that occur in nearly level drainage

basins. These soils are developed from fine-grained sediments that

are mainly of basaltic origin. They have dark brown, platy,

medium-textured A horizons; mottled, brown, prismatic, clayey B

horizons; mottled, loamy C horizons; and consolidated, brittle Dr

horizons. These soils are moderately slow in permeability, and have

slow internal drainage (U.S.D.A., 1960).

The Wilkens series occurs almost entirely as an inclusion in

Ukiah soils (Strickler, 1965). Both have developed from similar

parent materials but the Wilkens is less stony. The Wilkens soils

are usually wet at least half of each year, and they have a very dense

vegetal cover. Erosion is normally slight except in water channels
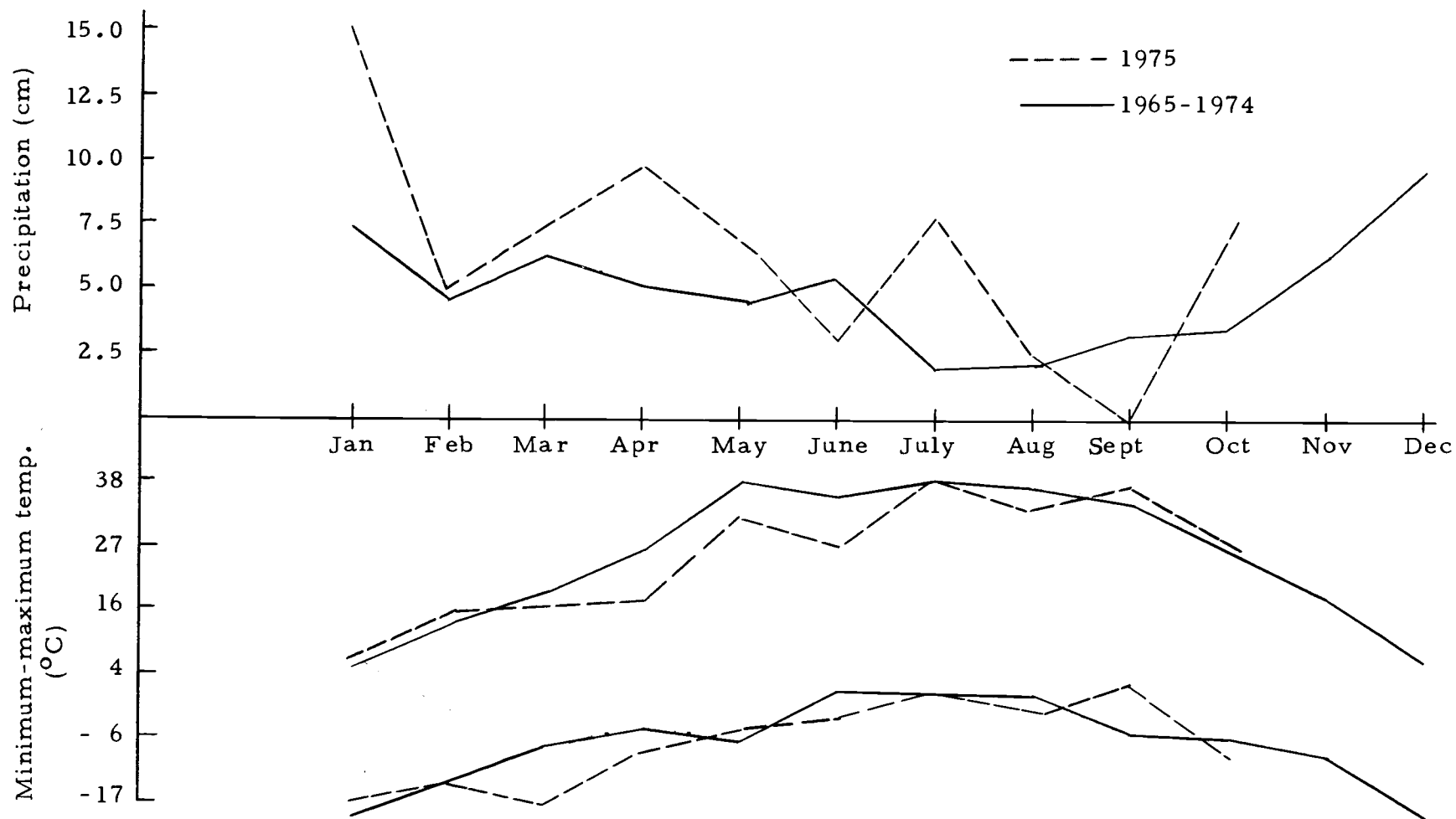
Figure 2. A comparison of precipitation and temperature deviation between a ten year average and 1975 on the study area.

where gullying often occurs. A description of the soil profile for the Wilkens series is found in Appendix B.

Vegetation

The study area was located on a dry meadow (Figure 3) approximating the description of Hall (1973). The area supported a beauty cinquefoil/velvet lupine - Kentucky bluegrass/timothy (Potentilla gracilis/Lupinus leucophyllus - Poa pratensis/Phleum pratense) community. Soil moisture was sufficient in the spring to support such forbs as common camas (Camassia quamash), piper anenome (Anenome piperi), smallflower woodlandstar (Lithophragma parviflora), longleaf eveningprimrose (Oenothera subacaulis), and swamp saxifrage (Saxifraga integrifolia). As the season progressed and soil moisture decreased, other species became more visible. Among these were yarrow (Achillea millefolium), pale agoseris (Agoseris glauca), autumn willowweed (Epilobium paniculatum), field horsetail (Equisetum arvense), velvet lupine, gland cinquefoil (Potentilla glandulosa), beauty cinquefoil, Oregon checkermallow (Sidalcea oregana), Missouri goldenrod (Solidago missouriensis), and yellow salsify (Tragopogon dubius).

Other forbs included western pasqueflower (Anenome occidentalis), rose pussytoes (Antennaria microphylla), orange arnica (Arnica fulgens), shaggy fleabane (Erigeron pumilus), blueleaf
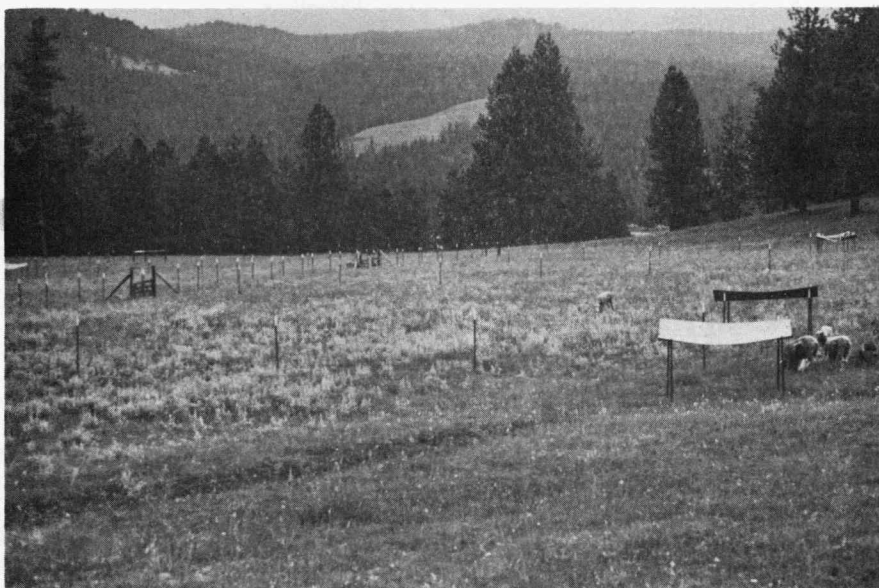
Figure 3.   Overall view of the study area on the Hall Ranch of the Eastern Oregon Agricultural Research Center.

strawberry (<u>Fragaria</u> <u>virginiana</u>), northern bedstraw (<u>Galium</u> <u>boreale</u>), sticky geranium (<u>Geranium</u> <u>viscosissimum</u>), prairiesmoke avens (<u>Geum</u> <u>triflorum</u>), Rockymountain iris (<u>Iris</u> <u>missouriensis</u>), sheep sorrel (<u>Rumex</u> <u>acetocella</u>), common dandelion (<u>Taraxacum</u> <u>officinale</u>), American vetch (<u>Vicia</u> <u>americana</u>), and hook violet (<u>Viola</u> <u>adunca</u>).

Kentucky bluegrass, as the most visible grass, shared dominance with timothy. Other graminoids on the study area included smooth brome (<u>Bromus</u> <u>inermis</u>), soft brome (<u>Bromus</u> <u>mollis</u>), California danthonia (<u>Danthonia</u> <u>californica</u>), Idaho fescue (<u>Festuca</u> <u>idahoensis</u>), Baltic rush (<u>Juncus</u> <u>balticus</u>), prairie junegrass (<u>Koeleria</u> <u>cristata</u>), Canada bluegrass (<u>Poa</u> <u>canadensis</u>), and western needlegrass (<u>Stipa</u> <u>occidentalis</u>). Several sedges (<u>Carex</u> sp.) were found but not identified to species.

The only shrub growing on the meadow was common snowberry (<u>Symphoricarpos</u> <u>albus</u>) which assumed a rather low growth form. This character plus its scattered distribution made it an inconspicuous component of the flora on the study area. A complete list of plant species growing on the study area is presented in Appendix C.

Land Use History

According to Hug (1961), settlement of the Union area began in the middle 1860's. Catherine Creek canyon provided a natural passageway from the Grande Ronde Valley to the mountain rangelands

of the western Wallowas. The Hall Ranch served as spring and fall range for cattle and sheep. Horses were wintered on lower meadows. Experiment Station records show that the Hall Ranch was heavily grazed by cattle and sheep from 1936 to 1956. A range condition survey in 1956 classified much of the Hall Ranch as poor condition. During the summer of 1974 a range condition survey was undertaken by the Soil Conservation Service. Many of the sites formerly classified as poor condition were reclassified as fair or good condition. The study area was classified as fair condition.

Keniston (1957) reported that much of the Hall Ranch was logged in the 1930's, but similar cuttings had occurred at earlier intervals. The remains of three sawmills are found on the Hall Ranch and the foundations of 11 others within a mile of the ranch's boundaries (Young, 1965).

REVIEW OF THE LITERATURE

Esophageal Fistulation

The literature is replete with information concerning the application of esophageal fistulation to food habits research. There are two excellent reviews of the subject to which the reader is referred: Van Dyne and Torell (1964) and Rice (1970).

The technique is not a recent development as attested to by Van Dyne and Torell (1964) who reported three early researchers that used esophageal fistulation: Magendie and Ryer in 1847, and Claude Bernard in 1855 fistulated horses, and in 1889 Pavlov installed esophageal fistulae in dogs. However, it has only been in the last two decades that the technique has been widely applied to ruminants (Van Dyne and Torell, 1964).

Surgical Procedures

A variety of surgical procedures has been reported. Notable among these are Torell (1954), Cook et al. (1958, 1963); Hamilton et al. (1960); Lesperance et al. (1960a, b); McManus et al. (1962); Chapman and Hamilton (1962); Van Dyne and Torell (1964); and Harris et al. (1967). The reader is referred to these for complete descriptions of surgical technique. At best, surgery of the esophagus is difficult; this accounts for numerous losses and for skepticism

regarding the technique (Van Dyne and Torell, 1964). Rice (1970)

reports the following difficulties associated with esophageal fistula-

tion: the esophagus does not have an enclosing serosa; it has a poor

blood supply; the fistulation restricts the necessary movement of the

esophagus; scar tissue often forms restricting functioning of the

musculature; food blockage often occurs, and animals will die if it is

not removed; the cannula may be expelled or swallowed; and extensive

salivary loss and serious dehydration may occur within a few hours.

Closure Devices

Numerous devices for closing esophageal fistulae have evolved

with the technique. Torell (1954) attempted to close the fistula by

tying bandages around the neck with the use of elastic bands, but

abandoned this procedure when he found that it impaired peristaltic

movements of the esophagus and led to the death of the animal.

Torell (1954) reported a technique whereby polyethylene tubing

was imbedded into the subcutaneous fascia longitudinally on both sides

of the fistula. The tubing acted as permanent channels for stainless

steel pins. When these pins were inserted and the protruding ends

tied together with elastic bands, the fistula could be effectively closed.

Hamilton et al. (1960), however, used this technique and found it to

be unsatisfactory, as leakage could not be controlled. Torell (1954)

developed a plug for closure of esophageal fistulae by inserting a

plastic plate which remained on the outside of the fistula. This device and several esophageal cannulae are illustrated in Van Dyne and Torell (1964), and Harris et al. (1967).

Other developments in closure devices include the lucite, acrylic plastic or stainless steel nonremovable cannulae used by Cook et al. (1958), Lesperance et al. (1959, 1960b), Rusoff and Foote (1961), and others. The exact dimensions of these cannulae vary with the type of animal and the desires of the researcher, but the basic design of the device is "T" shaped. A flanged end is seated in the esophagus itself, and the attached cylinder protrudes from the fistula. Closure of the cannula is usually accomplished with a screw cap or rubber stopper. Nonremovable cannulae have at least two disadvantages: 1) the anterior lip of the flange may protrude through the esophagus and skin (Lesperance et al., 1960b) or may cause the formation of a blind pouch anterior to the fistula and eventually may be expelled (Van Dyne and Torell, 1964), and 2) they have a tendency to become plugged with forage during sampling periods, thus reducing sample size (W. C. Krueger, personal communication). Hamilton et al. (1960) compared the amounts of ingesta recovered from a completely open fistula to that recovered through a rigid nonremovable cannula, and found that 15 to 33% more recovery was possible with the completely removable plug. The rigid, nonremovable lucite cannula, however, has some advantage in cold weather sampling

because it can be used while the operator is wearing gloves (Van Dyne and Torell, 1964).

Recovery of Samples

Lesperance et al. (1974) reported esophageal fistula samples that are less than total recovery of the actual amount of forage consumed may result in erroneous botanical composition of diets. These same workers further reported that complete collection of ingested materials probably never occurs. Several factors appear to influence recovery rate through esophageal fistulae, including: feed density, presence or absence of a cannula, cannula size, and perhaps species of animal (Lesperance et al., 1974).

In a chemical evaluation of grazed forages, Lesperance et al. (1960a) reported less crude fiber and more crude protein of alfalfa hay from esophageal fistulae than from the corresponding hay before sampling. Since these workers determined that alfalfa leaves are lower in crude fiber and higher in crude protein than alfalfa stems, they concluded that the more fibrous portions of the boluses continued down the esophagus while the less fibrous parts were collected. Campbell et al. (1968) collected forage through nonremovable cannulae in beef cattle. Recovery of organic matter fed to these animals varied from 84% to 94% for concentrates, and from 26% to 81% for roughages. These workers reported that plugging of the cannulae

was the main cause of lower recovery. In a study utilizing non-

removable esophageal cannulae, Kiesling et al. (1969) reported

recovery of concentrates to be similar to those of Campbell et al.

(1968) but considerably higher than those for roughages. Thus, it

would seem that high fiber diets, and the presence of permanent

cannulae may increase the recovery problem (Lesperance et al.,

1974). The amount of ingesta recovered from nonremovable esopha-

geal cannulae varied with the size of the opening (Van Dyne and

Torell, 1964). It appeared that the minimum size of cannula in

cattle would be 50 mm (Lesperance et al., 1974). Inside openings in

cannulae are usually about 3 cm in diameter in sheep (Van Dyne and

Torell, 1964), however, cannula size has not been adequately

investigated in sheep (Lesperance et al., 1974).

Grazing Behavior of Fistulated Animals

It is difficult to evaluate whether the grazing behavior of

esophageal-fistulated animals differs quantitatively from that of non-

fistulated animals (Van Dyne and Torell, 1964). While some

researchers have reported that successfully fistulated animals graze

normally (Arnold et al., 1964), others question whether fistulated

animals graze in the same manner as intact animals (Engles and

Malan, 1973).

Sampling Size

Literature suggesting the number of esophageal fistula samples required to estimate the diets of grazing animals for various vegetation types is scarce. Harniss et al. (1975) reported that while sample size varies with dietary species composition, probability level, and confidence interval, six sheep over six days would probably be minimal to adequately sample sagebrush-grass range. Van Dyne and Heady (1965), working in an open oak-annual grassland, suggested that at least five animals are required to sample dietary botanical composition, and as many as nine would be necessary for sampling within 10% of the mean with 90% confidence. Heady and Torell (1959) used three sheep in each of five sampling periods to determine forage preferences on annual grassland. Laycock et al. (1972) used seven sheep in three sampling periods to estimate diets in the tall-forb type of southwestern Montana. Rice et al. (1969) collected nine esophageal fistula samples to determine the diets of sheep grazing blue gramma (Bouteloua gracilis) range in Wyoming.

Microtechniques and Fecal Analysis

Application of Microtechniques

The application of microtechniques and plant histology to food habits research was pioneered by Baumgartner and Martin (1939) who

found gross analysis of ingesta unsatisfactory for squirrels which finely masticated their food. Dusi (1949, 1952) developed procedures for the adaptation of histological analysis to the feces of rabbits and the stomach contents of mice. Martin (1955) qualitatively determined the diets of sheep based upon characteristics of leaf cuticle in feces. Crocker (1959) applied this method to sheep grazing tussock grasslands. Hercus (1960) identified the diets of free-grazing sheep in New Zealand using Martin's (1955) procedures. Storr (1961) determined the diets of Quokkas by microscopic identification of the epidermis of leaves and stems of plants recovered in the feces. Other workers have utilized microtechniques to determine the diets of numerous animals (Table 1).

Some workers have adapted microtechniques to evaluate the dietary overlap of herbivores. Casebeer and Koss (1970) compared the relative selectivity of grasses by wildebeest, zebra, hartebeest and cattle in Kenya. Hansen and Reid (1975) described the seasonal diets of deer, elk and cattle using the same range. Hansen, Peden, and Rice (1973) measured the degree of dietary overlap of cows, bison and sheep.

Utility of Fecal Analysis

Proponents of the application of microtechniques to food habits research explain the popularity of the method on the basis of its

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Table 1. Studies employing microtechniques to determine diets.

Species	Reference
Grasshoppers (Phoetaliotes sp.)	Mulkern and Anderson, 1959
	Brusven and Mulkern, 1960
	Mulkern et al., 1962
	Pruess, 1969
	Mulkern et al., 1969
	Hansen and Ueckert, 1970
Mormon crickets (Anabrus simplex)	Hansen and Ueckert, 1970
Millipeds (Glomerus sp.)	Williams, 1969
Chyckwalla (Savromalus obesus)	Hansen, 1974
Kangaroo rats (Dipodomys merriami)	Soholt, 1973
Mountain pocket gopher (Cratogeomys castanops)	Ward, 1970
Northern pocket gopher (Thomomys talpoides)	Ward and Keith, 1962
	Vaughn, 1967
Plains pocket gopher (Geomys bursarius)	Myers and Vaughn, 1965
Ground squirrels (Citellus richardsonii)	Hansen and Veckert, 1970
Opossum (Trichosurus vulpecula)	Dunnet et al., 1973
European hare (Lepus europaeus)	Williams, 1969
Rabbit (Lepus sp.)	Hansen and Flinders, 1969
Black-tailed jackrabbit (Lepus californicus)	Hayden, 1966
	Sparks, 1968
	Hansen, 1972
White-tailed jackrabbit (Lepus townsendii)	Bear and Hansen, 1966
Waterbuck (Kobus defasa)	Kiley, 1966
Wildebeest (Connochaetes taurinus)	Stewart, 1967
Coke's hartebeest (Alcelaphus buselaphus)	" "
Grant's gazelle (Gazelle granti)	" "
Thomson's gazelle (Gazelle thomsonii)	" "
Buffalo (Syncerus caffer)	" "
Steinbuck (Raphicerus campestris)	" "
Common zebra (Equus burchellii)	" "
Elk (Cervus canadensis)	Ward, 1970
	Karfhage, 1974
Pronghorn antelope (Antilocapra americana)	Jacobs, 1973
Horse (Equus caballus)	Hansen, 1976
Mule deer (Odocoileus hemionus)	Kufeld et al., 1973
	Goodwin, 1975
	Hansen and Dearden, 1975
Bison (Bison bison)	Peden et al., 1974

(Continued on next page)

Table 1. (Continued)

Species	Reference
Bighorn sheep (Ovis canadensis)	Todd and Hansen, 1973
	Todd, 1975
Cattle (Bos tarus)	Vavra et al., 1970
	Rosiere et al., 1975
Newfoundland caribou (Rangifer tarandus)	Bergrund and Russell, 1964
Red deer (Cervus elaphus)	Burckhardt, 1959
	Hegg, 1961
Roe deer (Capreolus capreolus)	Burckhardt, 1959
	Hegg, 1961
Chamois (Chamois sp.)	Burckhardt, 1959
	Hegg, 1961

utility. Fecal analysis allows practically unlimited sampling and

requires fewer samples than rumen analysis (Anthony and Smith,

1974). Fecal analysis does not interfere with the normal habits of

the animal as may occur with the use of harnesses and fistulae, and

it imposes no restrictions on the movement of the animals, and is of

particular value where animals range extensively over mixed com-

munities (Crocker, 1959). Fecal analysis is the only feasible pro-

cedure to use when studying secretive and/or endangered species

where observations and/or rumen collections cannot be carried out

(Anthony and Smith, 1974). Fecal analysis is advantageous when two

or more animals are utilizing the same range (Korfhage, 1974).

The basis of the microtechnique is that fragmentary material

in the feces bears sufficient characteristic features to be identifiable

as belonging to a specific plant (Crocker, 1959). The most consistent

and useful characteristics were found by Stewart (1965) to be the form

of the silica bodies, the presence or absence and form of micro-

hairs and papillae, the appearance of the base of macro-hairs and of

the accompanying specialized epidermal cells, the shape and distribu-

tion of the stomata, and the appearance of the walls of the intercostal

long cells.

Basic diagnostic features of the epidermis of grasses were

exhaustively discussed by Metcalfe (1960). Keys to the identification

of plant fragments based on epidermal characteristics have been

developed for East African plains grasses (Stewart, 1965, 1967) for

selected species of the Gir Forest of India (Satakopan, 1972), for

selected range plants of eastern Oregon (Schrumpf, 1968), for plants

important to elk of the Blue Mountains of northeastern Oregon

(Korfhage, 1974), and for plants eaten by rangeland grasshoppers

(Brusven and Mulkern, 1960). The unique epidermal characteristic

of other species have been described (Davies, 1959; Hansen et al.,

1971).

Collection and Storage of Samples

Collection and storage of samples can play an important part in

the success of a study (Ward, 1970). For fecal analysis, collection

must be restricted to fresh fecal materials to prevent destruction of

plant parts by insects, bacteria, and fungi (Ward, 1970). For practi-

cal reasons, he suggested that sample data collected from many

different fecal groups were more valuable than detailed examination

of all materials in one dropping. By mixing the fecal material and

picking random subsamples, a representative sample can be obtained

in the field. The fecal material should be stored in plastic bags to

prevent drying and can be refrigerated to insure freshness and avoid

hardening and molding (Ward, 1970). Giles (1969) reported that

freezing has the special advantage of preserving both color and texture

and is probably the most convenient and practical method of preserva-

tion.

Hercus (1960) and Williams (1969) preserved feces in mixtures of alcohol, formalin, and acetic acid (A.F.A.). Hegg (1961) stored fecal pellets by simply air-drying. Hansen et al. (1971) preferred all dietary samples from large herbivores (all stomach, fistula, rumen or fecal material) be air dried and ground in a laboratory mill through a 1 mm (20 mesh) screen to reduce all particles to a uniform size.

Preparation of Microscope Slides

Several methods for the preparation of material for microscopic examination have been introduced. Each involves separation and clearing of plant fragments prior to mounting them on microscope slides.

Baumgartner and Martin (1939) immersed small amounts of stomach contents in Hertwig's clearing solution on microscope slides to prepare temporary mounts. Dusi (1949, 1952) used alcohol to disperse fecal material on microscope slides before adding chloral hydrate solution. Crocker (1959) simply diluted fecal samples with water and spread the material between two microscope slides prior to examination. Special staining treatments were unnecessary since the action of the digestive juices darkened the cellular tissues and imparted a light brown stain to the cuticle.

Hercus (1960) made suspensions of 3-gram samples of fecal material in 100 ml of water. Three subsamples were then withdrawn

for microscopic examination with no further preparation. Storr (1961) mounted fecal samples in euparal after first treating the fecal material by drying, grinding, boiling in a mixture of nitric and chromic acids, washing in water, staining with gentian violet and centrifuging. After softening air-dried fecal pellets in water, Hegg (1961) heated the material for 5 minutes in 10% potassium hydroxide. The solution was then shaken to loosen the epidermis, and decanted. Plant fragments were then stained and mounted in glycerin gelatin. Stewart (1967) heated fecal material in nitric acid to clear the epidermal fragments. The sample was then made up to 100 ml with water, boiled, stirred and spread on microscope slides.

Zyznar and Urness (1969) treated fecal pellets by soaking overnight or boiling in 10% sodium hydroxide for 15 minutes. The solution was then vigorously stirred to reduce the pellets to a pulpy mass. This material was allowed to settle, removed from the supernatant fluid, rinsed, and examined wet. Casebeer and Koss (1970) crushed subsamples of fecal pellets in 20 ml of water and 4 ml of concentrated nitric acid. After soaking for 24 hours, the volume was made up to 45 ml and boiled for 20 minutes. The solution was then mixed with xylol, washed in a sieve, and spread on a microscope slide. Ward (1970) stirred fecal material in water and detergent prior to filtering through silk bolting cloth. The samples were then placed on

microscope slides and spread in Hoyer's solution before being covered with a coverslip.

Hansen et al. (1971) stated that all dietary samples should be ground in a Wiley laboratory mill through a 1 mm (20 mesh) screen before microscope slides are made. The sample should then be washed with water for 1 minute on a 0.1 mm (20 mesh) screen. A small amount of this material is then spread on a microscope slide. Three or four drops of Hertwig's solution are then added to the sample and heated over a Bunsen burner. When most of the Hertwig's solution has boiled off, a few drops of Hoyer's solution are added, the sample spread evenly over the slide, and a coverslip placed on the preparation. The slide is then heated over a burner until the mixture begins to boil. The bottom surface of the slide is then wiped with a cold, damp sponge to draw air bubbles out of the Hoyer's solution. A teasing needle is then used to gently press on top of the coverslip to squeeze out excess mounting medium. Slides are then dried at 55°C for two to three days.

Korfhage (1974) blended fecal pellets for one minute with water in a Waring blender. The sample was then washed on a 200 (0.1 mm) mesh screen and stored in 95% ethyl alcohol. Subsamples were withdrawn, placed in water, mixed in a blender for several seconds, washed on a 200 mesh screen and mounted on microscope slides in glycerine jelly.

Preparation of Reference Material

The first requirement in a diet study using microscopic analysis is a reference collection of epidermal tissues from food plants in the study area (Storr, 1961). Reference slides must be prepared from the different parts of each plant including stems, leaves, flowers, and roots (Hansen et al., 1971).

A variety of procedures for preparing epidermal tissues for voucher specimens has been described. A thorough review of the literature concerning this type of work prior to 1968 is presented by Schrumpf (1968). Crocker (1959), Hercus (1960), and Storr (1961) separated epidermis from underlying plant tissues using acid solutions. Davies (1959), Metcalfe (1960), and Ward (1970) used scraping methods. Other workers have made replicas of plant surfaces using Archer adhesive (Sinclair and Dunn, 1961), silicone rubber products (Shutak and Dayawon, 1966), and cellulose acetate (Stoddard, 1965).

Processing time has been reduced by the introduction of new techniques. Sparks and Malechek (1968) first ground oven dried plant materials over a 1 mm screen, then washed them over a 200 mesh screen to insure mixing, to remove dirt, and to remove very small plant fragments. Small portions of the mixture were then spread evenly and mounted on microscope slides using Hertwig's solution and Hoyer's solution. Korfhage (1974) ground leaf fragments in warm

water at high speeds in a Waring blender. Blending times depended
on leaf thickness, but one minute was usually sufficient to separate
both surfaces. Following this treatment, epidermal fragments were
roughly equivalent to those found in fecal material. Several epidermal
fragments were removed from the blended solution with the aid of a
camel hair brush, and floated in several drops of water onto a micro-
scope slide. Epidermal surfaces were permanently mounted on
microscope slides using Hertwig's solution followed by a small block
of glycerine jelly.

Quantification of Dietary Components

Early applications of microtechniques to food habits research
were largely qualitative. An initial attempt to quantify the food habits
of herbivores was made by Adams (1957), who compared the number
of identifiable fragments in the feces of captive hares to the actual
weight of food eaten for each species of plant in the diet. He further
postulated that such conversion factors could be applied to feces of
free-living animals to estimate the amounts of plants consumed.
Hercus (1960) speculated on the same idea when she stated that
quantitative estimates of intake and utilization would be dependent
upon establishing relationships between amounts of cuticle in the feces
to the actual amounts of each plant species eaten, either in terms of
weight or numbers of leaves. Adams et al. (1962) tested this

procedure by feeding captive hares known weights of plants and counting the numbers of identifiable plant fragments in the resultant feces. By regression analysis they demonstrated statistical significance between weight and number of identifiable fragments for only one of two plant species fed.

Kiley (1966) simply recorded the presence or absence of individual species' epidermis in the feces of waterbuck. Stewart and Stewart (1970) determined the relative frequencies of plant species by identifying a total of 100 epidermal fragments for each sample. Hercus (1960) counted the number of cuticle fragments of every species occurring on microscope slides. She found some variation in the total count of duplicate samples because the cuticle fragments were not even size or shape. Methods attempting to estimate the proportions of grasses ingested by counting all epidermal fragments were shown by Stewart (1967) to be invalid. This invalidation occurred because some of the species broke into smaller fragments and the total number of fragments was thus more numerous.

Other workers have attempted to quantify diets by estimating the area of epidermal fragments. Storr (1961) identified epidermal fragments and estimated the area of each species present in hundredths of a square millimeter by using a graduated eyepiece. Areas of epidermis were converted to weight equivalents of foliage and proportion of each item in a sample was calculated on a dry weight basis.

Stewart (1967) analyzed diets by measuring the area of 100 fragments in each of three fecal samples. The variance among duplicate samples was high due to the presence of occasional, very large fragments, and because of differential separation of fragments before or after these were placed on the slide.

The problem of having many different sizes of food particles in dietary samples was overcome by Malechek (1966) who first ground oven-dried rumen samples in a Wiley laboratory mill over a 1 mm screen. The botanical composition of each sample was determined by the relative number of epidermal fragments of each species recognized in 100 microscope fields. Sparks (1968) used a similar method to determine the diets of black-tailed jackrabbits in Colorado. Dried stomach contents were ground in a Wiley mill over a 20 mesh screen and washed over a 200 mesh screen to insure mixing and to remove dirt and very small fragments. The percentage of each food item in the diet was estimated by examining 20 systematically located fields on each slide. Average frequency percentages were computed for all species and converted to density per field.

Sparks and Malechek (1968) accurately estimated percent composition by dry weight for 15 mixtures of plants that are found in the diets of some small herbivores. The mixtures were sampled by recording the frequency of occurrence of each species in 100 microscope fields using 125-power magnification, converting to density,

and calculating relative density as an estimate of percent composition

by dry weight. Dry weight percentages were predicted directly from

relative density. The authors concluded that the microtechnique they

used would be an accurate means of determining the dry weight

composition of stomach samples, esophageal samples, rumen samples

and clipped herbage.

Ocular-Estimate-by-Plot

In this method, an estimate is made of the amount of herbage

removed by grazing (Brown, 1954). The technique was developed by

Pechanec and Pickford (1937a) and has been described and examined

critically by these authors. Estimates are made on plots located

either in a gridiron or patternized arrangement (Pechanec and

Pickford, 1937a).

Training is an important first step in employing the technique.

Brown (1954) presented the following description of this training:

> . . .the herbage on a small plot is clipped to simulate grazing
> (Sample A) and the percentage of herbage removed from the
> plot is estimated. The remaining herbage is then clipped
> (Sample B). Samples A and B are weighed separately.
> A = weight removed (utilization); A+B = total yield. Percent-
> age utilization = $\frac{A}{A+B}$ X 100. The estimated percentage of
> herbage removed is then compared with the actual percentage
> removed and the error determined. By means of estimates
> and checks each worker trains himself to recognize varying
> degrees of utilization.

Sampling Size

The most suitable number, size, shape, and manner of location

of plots is dependent upon the type of vegetation to be studied and

should be determined by trial before the study is initiated (Pechanec

and Pickford, 1937a). For studying utilization of key species on a

homogenous key area, 30 plots gave sufficiently precise results (Reid

and Pickford, 1941). In practice, plots are temporary or they may be

made permanent by some relocating device (Brown, 1954). Percentage

utilization is worked out from the average of estimates from the series

of plots, and is based on total yield (Brown, 1954).

Advantages and Disadvantages

Pechanec and Pickford (1937b) listed the following advantages of

the ocular-estimate-by-plot technique:

1) The method is reasonably accurate, and if frequent
   checks are made the degree of accuracy can be
   improved upon .
2) Estimates can be made rapidly, facilitating abundant
   replication of plots.
3) The method is widely adaptable to many different species,
   including grasses, weeds, and shrubs.

Inherent errors of the method have been described by several

workers. Smith et al. (1962) pointed out that considerable training is

necessary to achieve proficiency and observer bias can also affect

accuracy of data. The individual estimates tend to show less relative

dispersion than the actual quantities being estimated; that is, estimates

cluster around the mean, the error of estimate being positive for small

quantities and negative for large quantities (Pechanec and Pickford,

1937a). To get unbiased estimates, Smith (1968) weighted each

utilization estimate by production on the same plot.

Previous forage utilization is not always visible. Invisible

utilization may include plants that are pulled up by the roots, plant

parts that are pulled out leaving no visible stubble, deciduous fruits

or leaves, and use that has been obscured by subsequent growth

(Martin, 1970).

Application

The ocular-estimate-by-plot method has been used by several

workers to determine diets of grazing herbivores. Milles (1976)

determined the utilization of foothill rangelands by cattle and big game

employing this method. Laycock et al. (1972) reported that botanical

composition of sheep diets as determined by the ocular-estimate-by-

plot method gave slightly higher estimates of forbs and slightly lower

estimates of grasses than the esophageal fistula technique.

Stomach Content Analysis

Examination of rumen ingesta has been a widely used technique

to ascertain the diets of herbivores. An excellent review of the method was presented by Medin (1970).

Forbes and Bechdel (1931) were probably the first to apply this technique to wild ruminants. Numerous workers since have refined the technique and adapted it to their own needs (Rice et al., 1969; Casebeer and Koss, 1970; Field, 1972; Anthony and Smith, 1974; Eastman, 1974; Qvortrup and Blankenship, 1974).

It is often impractical to save the entire stomach contents of large herbivores, and a sample, commonly about a liter, is removed (Medin, 1970). Among ruminants, samples are generally taken from the rumen or rumen-reticulum. Contents are either mixed prior to sampling or samples are taken from several parts of the food mass (Medin, 1970).

The limitations of the technique have been discussed by several authors. The rumen contents of grazing animals contain the residue of certain forages eaten as much as ten days before sampling, whereas certain other components of the diet may remain in the rumen for only short periods (Rice, 1970). Thus, stomach analysis may be seriously biased toward more fibrous or less digestible materials in the diet. Norris (1943) reported wide variability between the composition of feeds in the rumen and that of the forage fed, and concluded that stomach analyses are of limited value in measuring the amounts of different forages eaten by sheep.

In a comparison of the stomach content analysis method and the esophageal fistula method, Rice et al. (1969) concluded that while the rumen sampling technique indicated the presence or absence of plant species, it could not be used to express quantitative relationships among plant species grazed where considerable variety was possible in the diet.

METHODS OF STUDY

Relative values of the esophageal fistula, stomach content analysis, fecal analysis, and ocular-estimate-by-plot methods were based on information collected from sheep grazing a tall-forb community, and confined sheep fed diets of known composition.

Summary of Approach

Microscope slide mounts were made from diet samples collected from the esophagus, rumen and feces of bi-fistulated[1] sheep grazing a beauty cinquefoil/velvet lupine - Kentucky bluegrass/timothy community during two, seven consecutive day periods. These slides were compared with material in a reference collection to determine kinds and amounts of plants in the animals' diets according to the methodology of Sparks and Malechek (1968) with appropriate modifications. Forage utilization measurements (Pechanec and Pickford, 1937b) were made immediately following each seven day grazing trial. Data were collected during two periods: early summer and late summer. Diets as determined by each method were converted to percent dry weight composition for comparison.

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[1] In this study, a bi-fistulated sheep is one with both esophageal and rumen fistulae.

In the second study phase ten confined bi-fistulated sheep were fed a hand-composited diet of known composition. Diet samples were collected from the esophagus, rumen, and feces of each animal. Percent dry weight composition of forage plants collected from each location was compared with every other location and with the original hand-composited diet to establish the accuracy of each technique.

Experimental Animals

The sheep used in this study were all mixed-bred yearlings. Fifteen of these were wethers and three were ewes. During the first rangeland grazing trial, 14 wethers and two ewes were utilized. The second rangeland grazing trial consisted of eight wethers and one ewe. During the feeding trial ten wethers were utilized. The variation in numbers of sheep utilized was the result of death loss.

Approximately two months prior to the collection of diet samples, esophageal fistulae were installed in all sheep according to the technique of Harris et al. (1967). Closure of these fistulae was accomplished by a stainless steel plate onto which was attached a removable rubber stopper (Figure 4).

Following surgery, all sheep were confined for three days, given free access to alfalfa pellets, water and salt, and injected daily with 10 cc of Combiotic® (penicillin dihydrostreptomycin in aqueous suspension). Following this confinement the animals were placed on
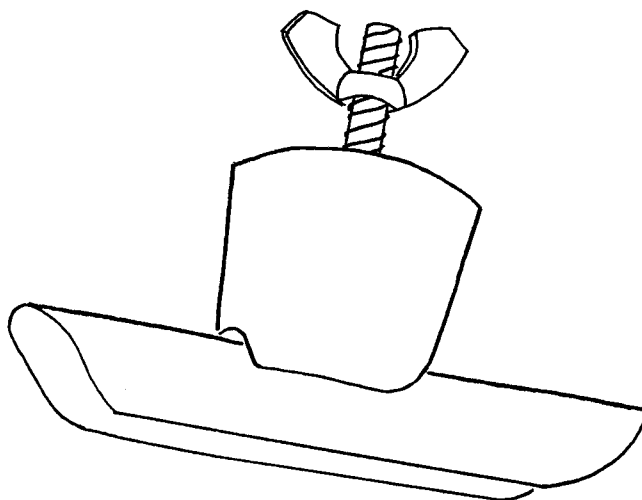
Figure 4. Stainless steel device used to close esophageal fistulae.
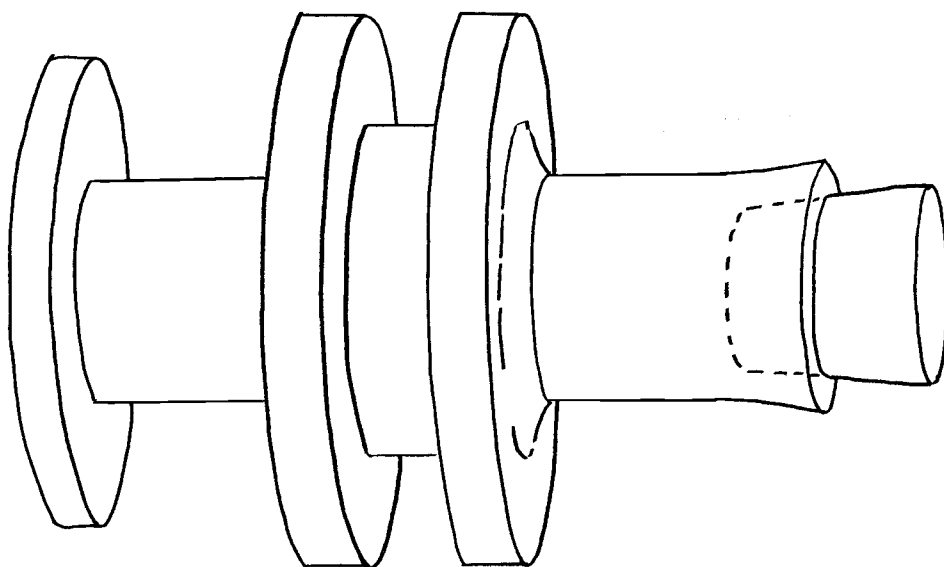
Figure 5. Rumen cannula utilized in this study.

green pasture and given free access to alfalfa pellets, water and salt.

Esophageal closure plates were removed weekly, cleaned in water,

coated with Furacin® ointment (nitrofurazone) and reinserted.

When the animals recovered from this operation, rumen fistulae

were installed in all sheep according to the procedure of McCann

et al. (1973). The closure device for rumen fistulae was a soft

rubber cannula (McCann et al., 1973). This type of cannula is a two-

piece unit consisting of a flanged tube held in place by a separate

doughnut-shaped piece and sealed with a rubber stopper (Figure 5).

Following surgery, the sheep were placed on green pasture and given

free access to alfalfa pellets, salt and water.

All sheep were shorn and paint branded prior to the first range-

land grazing trial. Wool in the immediate vicinity of esophageal and

rumen fistulae was clipped short to aid in the prevention of fly infesta-

tions. All animals were frequently dusted with fly repellent.

Rangeland Grazing Trials

Pasture Design

The 1.6 hectare study area was fenced so as to create a

0.8 hectare holding pasture and eight 0.1 hectare experimental

pastures (Figure 6). The fence was constructed of woven wire to

contain sheep while excluding coyotes. The holding pasture and
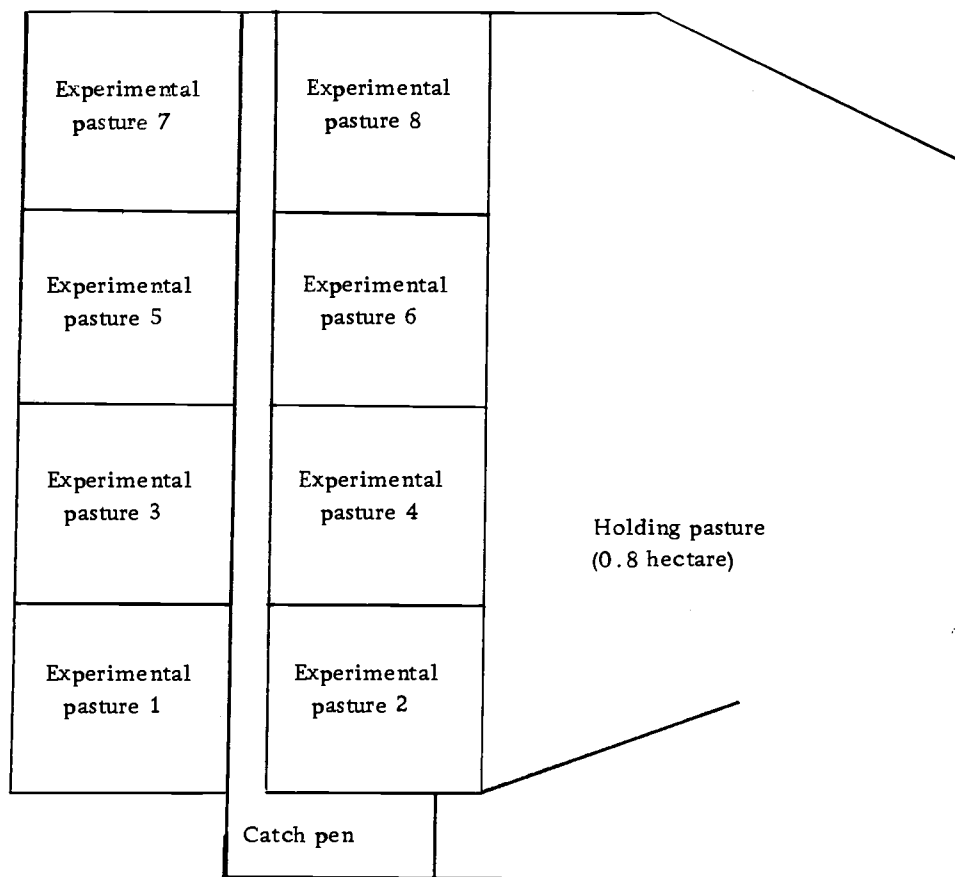
Figure 6. Pasture design for rangeland grazing trials. Each experimental pasture was 0.1 hectare in size and the holding pasture was 0.8 hectare.

experimental pastures were constructed on the same plant community

and the vegetation was virtually uniform throughout the area. A ply-

wood catch pen was constructed to facilitate the handling of animals

and the collection of samples.

Collection of Rumen and
Esophageal Ingesta

There were two rangeland grazing trials, each lasting for seven

consecutive days. The first trial was conducted from 5 July through

11 July (hereafter referred to as the early summer grazing trial) and

the second began on 19 August and ended on 25 August (hereafter

referred to as the late summer grazing trial). All sheep were main-

tained in the holding pasture (Figure 6) for five days prior to the initia-

tion of each grazing trial. This period allowed the animals to become

adjusted to the vegetation. At the end of this adjustment period, two

sheep were randomly assigned to each of the experimental pastures

(Figure 6) where the animals remained for the duration of the trial.

Since only nine sheep were studied during the second trial, two sheep

were randomly assigned to each of four experimental pastures, and

the ninth sheep was placed in an experimental pasture by itself.

Water, salt and shade were available to the sheep in each experimental

pasture at all times.

Prior to dawn of each morning, all sheep were removed from the experimental pastures and gathered in the catch pen (Figure 6). The sheep from each experimental pasture were captured, their esophageal closure plates removed, collection bags attached to their necks (Figure 7) and the animals were returned to the experimental pasture from which they had come. Fifteen minutes were allowed to pass before the second pair of sheep was captured, their esophageal plates removed, collection bags attached to their necks and they were returned to their respective experimental pasture.

At this time, the first pair of sheep had been allowed approximately one-half hour in which to graze. They were returned to the catch pen where the esophageal collection bags were removed, and the closure plates washed in water and reinserted into the esophageal fistulae. Ingesta which had been collected in the esophageal bags was placed in appropriately labeled plastic bags, sealed and set aside. Occasionally sheep ruminated while the esophageal fistula was open. When this occurred, rumen material fouled the esophageal ingesta sample. Only green material free of rumen odor was collected from the esophageal bags.

At this point, the rubber stopper sealing the rumen cannula (Figure 5) was removed and a pair of tongs used to collect rumen ingesta (Figure 8). The amount of material collected in this manner was variable but averaged approximately 10 grams on a dry weight
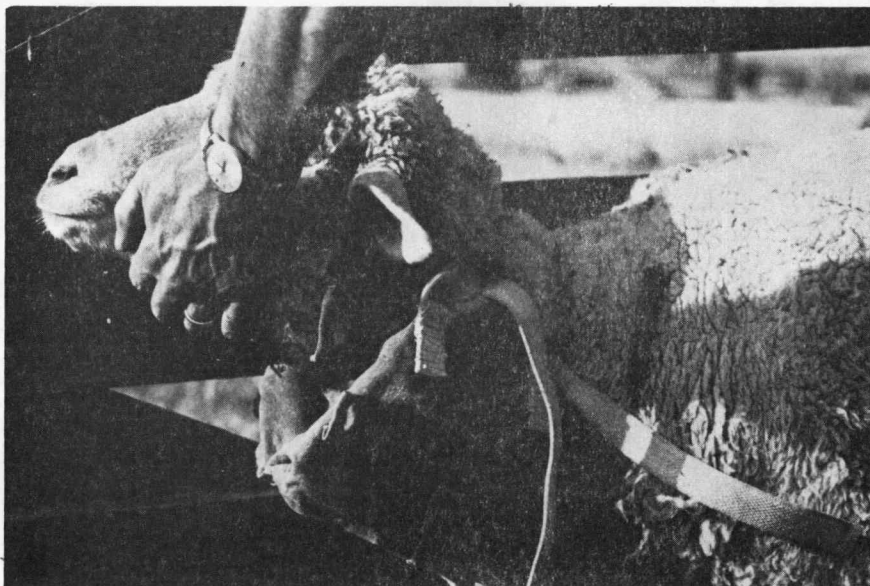
Figure 7. Esophageal collection bag in place.

Figure 8. Method used to collect rumen ingesta.

basis. An attempt was made to collect material from all portions of the rumen to avoid bias. After rumen material had been collected in appropriately labeled plastic bags, the rumen cannulae were resealed, and the sheep returned to the experimental pasture from which they had come.

This method of alternatively moving pairs of sheep to and from the catch pen facilitated handling and insured that all sheep were allowed approximately one-half hour in which to graze. When the animals were permitted to graze for longer than approximately one-half hour the incidence of fouled esophageal ingesta samples due to rumination increased. It was further noted that the muscles of the esophagus slowly contracted when the closure plate was removed, and reinsertion became increasingly difficult with the length of time the fistula was open. The effects of dehydration due to water loss through open fistulae were considered minimal as free water was constantly available.

Collection of Fecal Samples

Fecal material was collected from each experimental pasture every evening of both grazing trials. Occasionally pellets were collected from animals observed defecating. More frequently, how-ever, pellets were collected from the ground without knowledge of the specific animal which had deposited them. In the latter case, only

pellets with glossy mucous coatings, and free of insects were collected

to insure freshness. Pellets were avoided which were infested with

insects or showed such signs of weathering as dried, cracked or dull

surfaces. Several pellets from as many fresh pellet groups as could

be found within each experimental pasture were gathered, placed in a

plastic bag and sealed. Fecal material collected from observed

animals was mixed with fresh pellets gathered from the ground. By

collecting pellets from several groups, it was believed that a compo-

site sample of feces from both sheep within each experimental pasture

was attained. This method of collection had the distinct disadvantage

of prohibiting the estimation of diets for each sheep based on fecal

material. Furthermore, it is doubtful that a composite sample

yielded a true mean of the individual diets of the two sheep within any

given experimental pasture. It was not always possible to know

whether feces from each sheep were collected in a one-to-one ratio.

If the two animals in any given experimental pasture had different

diets, and fecal material from each sheep was not collected in a one-

to-one ratio, then a composite sample of feces would result in the

estimation of an erroneous mean diet. Fecal collection bags could

have been attached to each sheep to avoid the problems associated

with composite samples. It was believed, however, that the use of

fecal collection bags would have contributed excessive stress to the

animals. Fecal pellets could have been collected directly from the

rectum of every sheep at the same time esophageal and rumen

samples were collected. This method was also avoided because it

would have been irritating to rectal mucosa and time consuming.

Vegetation Measurements

Prior to each grazing trial, ten randomly selected 4.8 ft^2

circular plots were established in each of the experimental pastures

and protected with ten wire cages (Brown, 1954). The green weight of

each protected species was estimated (Pechanec and Pickford, 1937a).

Samples of each species were collected in the field, weighed with a

tubular gram scale, oven dried at 100°C for 48 hours and weighed

again to establish green weight to dry weight conversions. To mini-

mize the effects of plant growth while production was being estimated,

one plot from each experimental pasture was measured in sequence

rather than measuring all plots in one experimental pasture before

proceeding to the next pasture.

Utilization of herbaceous vegetation was determined by the

ocular-estimate-by-plot method of Pechanec and Pickford (1937b).

Immediately following each grazing trial all sheep were removed

from the study area. Ten 4.8 ft^2 circular plots were located in each

experimental pasture on a systematically random basis. Percent

utilization of current annual growth for each species was estimated.

Plots were randomly clipped to check for accuracy. To minimize the

effects of regrowth, one plot from each experimental pasture was measured in sequence rather than measuring all plots in one experimental pasture before proceeding to the next experimental pasture.

The phenological state of every species was estimated during both grazing trials by observing plants in each experimental pasture.

Feeding Trial

The objective of this portion of the study was to compare relative amounts of forage collected from the esophagus, rumen and feces with relative amounts of forage consumed. The assumptions made here were first that the relative amount of each forage species consumed was known and second that the relative amount of each forage species consumed remained constant throughout the feeding trial. In early July a field of ladino clover (Trifolium repens) and fawn fescue (Festuca arundinaceae) and a separate field of alfalfa (Medicago sativa) and orchardgrass (Dactylis glomerata) were mowed and the hay allowed to air dry. One hundred and thirty-six kilograms of each of these two mixtures were ground together in a Gehl 120 mobile grinder-mixer to reduce particle size and insure mixing, thereby eliminating the possibility of animals selecting particular species from the mixture. This forage was combined with enough molasses to reduce dust and enhance palatability, and fed ad libitum to ten bi-fistulated sheep maintained in ten separate stalls in a barn on the Eastern

Oregon Agricultural Research Center at Union, Oregon. Each sheep had free access to salt and water.

After a five day adjustment period on this forage, a feeding trial lasting seven consecutive days was conducted as follows: (1) in the morning of each day esophageal closure plates were removed from each sheep, every animal was outfitted with an esophageal collection bag and fed the mixture on an _ad libitum_ basis; (2) after about one-half hour of feeding, the bags were removed, their contents sealed in separate plastic bags, and the esophageal closure plates reinserted into the fistulae; (3) rumen cannulae were unplugged, ingesta was removed with tongs as previously described, and sealed in separate plastic bags, and the cannulae plugged; (4) fecal material was recovered from the rectum of each animal and sealed in separate plastic bags; (5) a grab sample of forage was collected from each of the ten stalls and sealed in separate plastic bags; (6) all forage was removed from the stalls each evening to prevent feeding at night and help insure feeding the next morning.

Frequency of occurrence of each species was recorded by reading three microscope slides per sample at the rate of 20 fields per slide. Percent composition of forage plants recovered from the esophagus, rumen and feces was compared with percent composition of forage plants in the original hand-composited diet to establish the accuracy of each technique.

Care of Samples

Following collection, samples were placed in plastic bags,
sealed and frozen. As time permitted, esophageal and rumen ingesta
samples were thawed, squeezed in cheesecloth to remove saliva and
rumen fluid, placed in paper bags and oven dried at $100^{o}C$ for 72
hours. Forage and fecal samples were thawed, placed in paper bags
and oven dried at $100^{o}C$ for 72 hours.

Upon drying, esophageal, rumen and forage samples were
ground separately in a Wiley laboratory mill through a 20-mesh (1 mm
openings) screen to reduce fragments to a uniform size (Sparks and
Malechek, 1968). These samples were stored in plastic bags until
preparation of microscope slides. Dried fecal material was stored
intact until microscope slides could be made.

Preparation of Microscope Slides

Esophageal, rumen and forage samples were subsampled by
taking approximately one gram of material. These subsamples were
individually placed in 50 ml beakers, covered with water and soaked
for a minimum of three hours. Following soaking, each subsample
was washed with water over a 200-mesh screen until filtrate was
clear. Each subsample was then placed in a semi-micro container
of a Waring blender and masticated for 90 seconds. Each subsample

was again washed over a 200-mesh screen, and a small amount

placed on a microscope slide. A few drops of Hertwig's clearing

solution[2] were added and the material spread out evenly on the slide.

The slide was then passed over the flame of a Bunsen burner until

most of the clearing solution had evaporated. When the slide was

cool, enough Hoyer's mounting medium was added to the material to

form a circle about the width of the slide. A 22 x 44 mm glass cover-

slip was placed over the sample and the slide was heated until the

mixture bubbled throughout. The slide was then cooled on a wet

sponge, excess material removed and the edges of the coverslip

sealed with additional Hoyer's mounting medium. The completed

slide was then oven dried at approximately $50^{\circ}C$ for 72 hours. Three

high quality microscope slides were prepared for each sample.

Preliminary experimentation indicated that fecal samples pro-

duced inferior microscope slides if first ground in the Wiley laboratory

mill. It was found that ground fecal material produced microscope

slides with fewer identifiable fragments than slides produced from

unground samples. Thus, fecal samples were treated differently than

esophageal, rumen and forage samples. Five pellets were randomly

selected from each sample, placed in a 50 ml beaker, covered with

water and soaked for several hours. The pellets were then masti-

cated in a Waring blender for 60 seconds, washed in water over a

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[2]Formulae for Hertwig's clearing solution and Hoyer's mounting
medium are given in Appendix D.

200-mesh screen and again soaked in water for one hour. Following this, fecal material was washed in water over a 200-mesh screen and microscope slides were prepared as previously described.

Reference Collection

A reference collection consisting of dried plants, microscope slides of epidermal tissue and photomicrographs was prepared to aid in the identification of plant fragments found in ingesta and forage samples.

Either dried or fresh plants were used in preparing microscope slides. Separate slides were prepared for sheath, stem, lamina, petiole, and inflorescence components where appropriate. Plants were treated in the following manner to prepare microscope slides: (1) plants were separated into their component parts and individually placed in 50 ml beakers and soaked in water for three hours; (2) the sample was then masticated in a semi-micro container of a Waring blender for 90 seconds, and washed over a 200-mesh screen; (3) two microscope slides of each component were then prepared following the previously described procedure.

Photomicrographs were taken of epidermal tissues using a Wild M20 binocular microscope with photo tube, Wild floutar objectives, Wild achromatic aplanatic condenser, Wild low voltage built-in microscope light with transformer, Wild intermediate shutter with

Format indicating eyepiece BK 10, and Kodak Pony II camera. Kodak

Panatomic X film (ASA 32) was used. Developing and printing of film

was done commercially following standard Kodak procedure.

Plant Identification

Three slides were prepared for each sample and each was

examined at the rate of 20 fields (systematically selected) at 100

power magnification using a Leitz binocular microscope and Bausch

and Lomb objectives. Blank fields were ignored. Identification of

plant species from dietary samples was based on comparison with

epidermal characteristics of reference material.

Quantification of Diets

The estimated percentage of utilization of each species was

multiplied by its average dry-weight production to estimate the

amount eaten (Laycock et al., 1972). The percent dry weight compo-

sition of each species was then calculated to enable comparisons with

the other techniques.

Data taken from microscope slide readings were expressed as

percent frequency (number of fields in which the species occurred per

100 fields) for every species identified in esophageal, rumen and fecal

samples. Percent frequency of each species was converted to density

using a table (Appendix E) developed by Fracker and Brischle (1944)

and described by Hansen and Flinders (1969). The relative density of

each species was determined on a percentage basis using the following

formula (Sparks and Malechek, 1968):

$$\text{Relative density} = \frac{\text{Density of fragments of species a}}{\text{Total density of fragments of all species}} \times 100$$

Percent dry weight composition of each species was assumed to be

the same as its calculated relative density (Sparks and Malechek,

1968).

Statistical Analysis

Two statistical methods were employed to aid interpretation of

data. These were two-factor analysis of variance followed by

Duncan's new multiple-range test (Steel and Torrie, 1960) where

applicable.

Grazing Trials

Data were subjected to two-factor analysis of variance using

randomized block design. Relative densities of plant species identi-

fied in esophageal, rumen and fecal samples were converted to per-

cent composition by dry weight (Sparks and Malechek, 1968) to enable

comparison with utilization data. Data from each treatment were

pooled separately for each experimental pasture. Data from each

treatment and each pasture were then averaged over days to yield a single mean for each treatment and each pasture. The outline of the analysis for the early summer grazing trial is as follows:

Source of variation	Degrees of freedom
Pastures	6
Treatment	3
Pastures x treatment	18
Total	27

The outline of the analysis for the late summer grazing trial is as follows:

Source of variation	Degrees of freedom
Pastures	4
Treatment	3
Pastures x treatment	12
Total	19

Treatment means were compared using Duncan's new multiple-range test (Steel and Torrie, 1960) at the 95% confidence level.

Feeding Trial

Data were subjected to two-factor analysis of variance using randomized block design. Relative densities of plant species identified in feed, esophageal, rumen and fecal samples were converted to percent composition by dry weight (Sparks and Malechek, 1968) for comparison. Data from each treatment were pooled separately for each sheep. Data from each treatment and each sheep were then averaged over days to yield a single mean for each treatment and each

sheep. The outline of the analysis is as follows:

Source of variation	Degrees of freedom
Sheep	9
Treatment	3
Sheep x treatment	27
Total	39

Treatment means were compared using Duncan's new multiple-range test (Steel and Torrie, 1960) at the 95% confidence level.

Similarity Index

The similarity between botanical composition of the diets as determined by each method was studied using Kulczynski's mathematical expression of similarity (Oosting, 1956). The similarity indices were calculated by the formula:

$$\frac{2w}{a+b} \times 100$$

where w is the lowest of the two values being compared, and a and b are the two values being compared.

Relative Preference Index

The preference of major plant species occurring in the diets during the rangeland grazing trials was determined with the following relative preference index (RPI) (Krueger, 1972):

$$RPI = \frac{Percent\ composition\ in\ diet}{Percent\ composition\ on\ range}$$

RESULTS AND DISCUSSION

Feeding Trial

The dry weight composition of orchardgrass, fawn fescue, alfalfa, and ladino clover of the control (hand-composited feed) and of diet samples collected from each sampling location (esophagus, rumen, and feces) was calculated separately. The dry weight composition of a composite of both grasses and a composite of both forbs was also determined for the control and for each sampling location.

Total Grass

There was no significant difference between the amount of grass in the control and in esophageal ingesta samples (Figure 9). Rumen samples contained significantly less grass than fecal samples, but significantly more grass than either the control or esophageal samples. The amount of fecal samples was significantly higher than in any of the other sampling locations.

Total Forbs

No significant differences occurred between the amounts of forbs in the control and in esophageal ingesta samples (Figure 9). Rumen samples contained a significantly lower forb content than did the
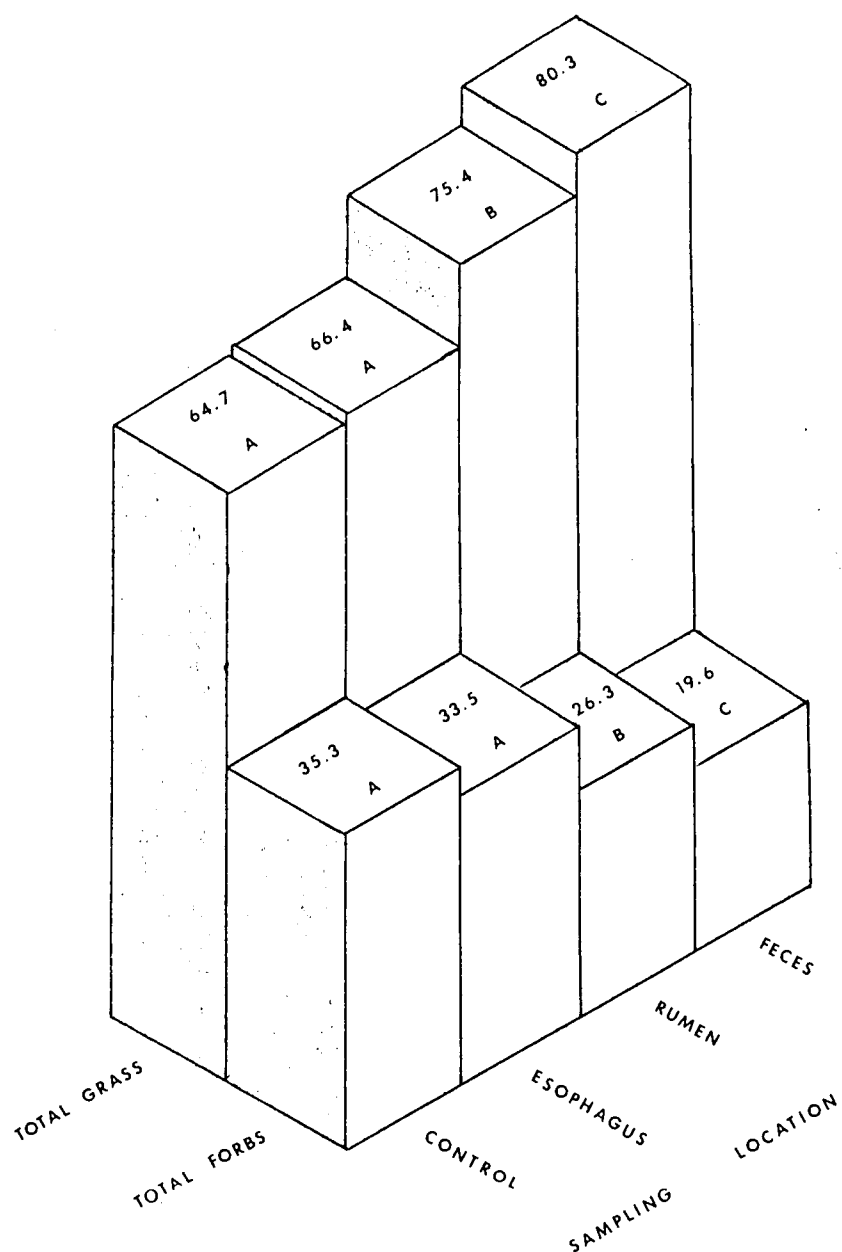
Figure 9. Percent dry weight composition of all grasses and all forbs in each sampling location. Treatments with the same letters within vegetative types are not significantly different at the 95 percent confidence level.

control or samples of esophageal ingesta. Fecal samples contained significantly fewer forbs than any other sampling location.

Fawn Fescue

There was no significant difference between the amount of fawn fescue in esophageal ingesta samples and that in the control (Figure 10). While there was no significant difference between the amounts of this grass in fecal and rumen samples, both of these sampling locations contained significantly more fawn fescue than the control or esophageal samples.

Orchardgrass

The amount of orchardgrass in fecal samples was significantly higher than in any other sampling location (Figure 10). There was no significant difference among the amounts of this grass in the control, esophageal samples, and rumen samples.

Alfalfa

Fecal samples contained significantly lower amounts of alfalfa than other sampling locations (Figure 10). The amount of rumen ingesta was not significantly different than that contained in esophageal samples, but was significantly lower than in the control. There was
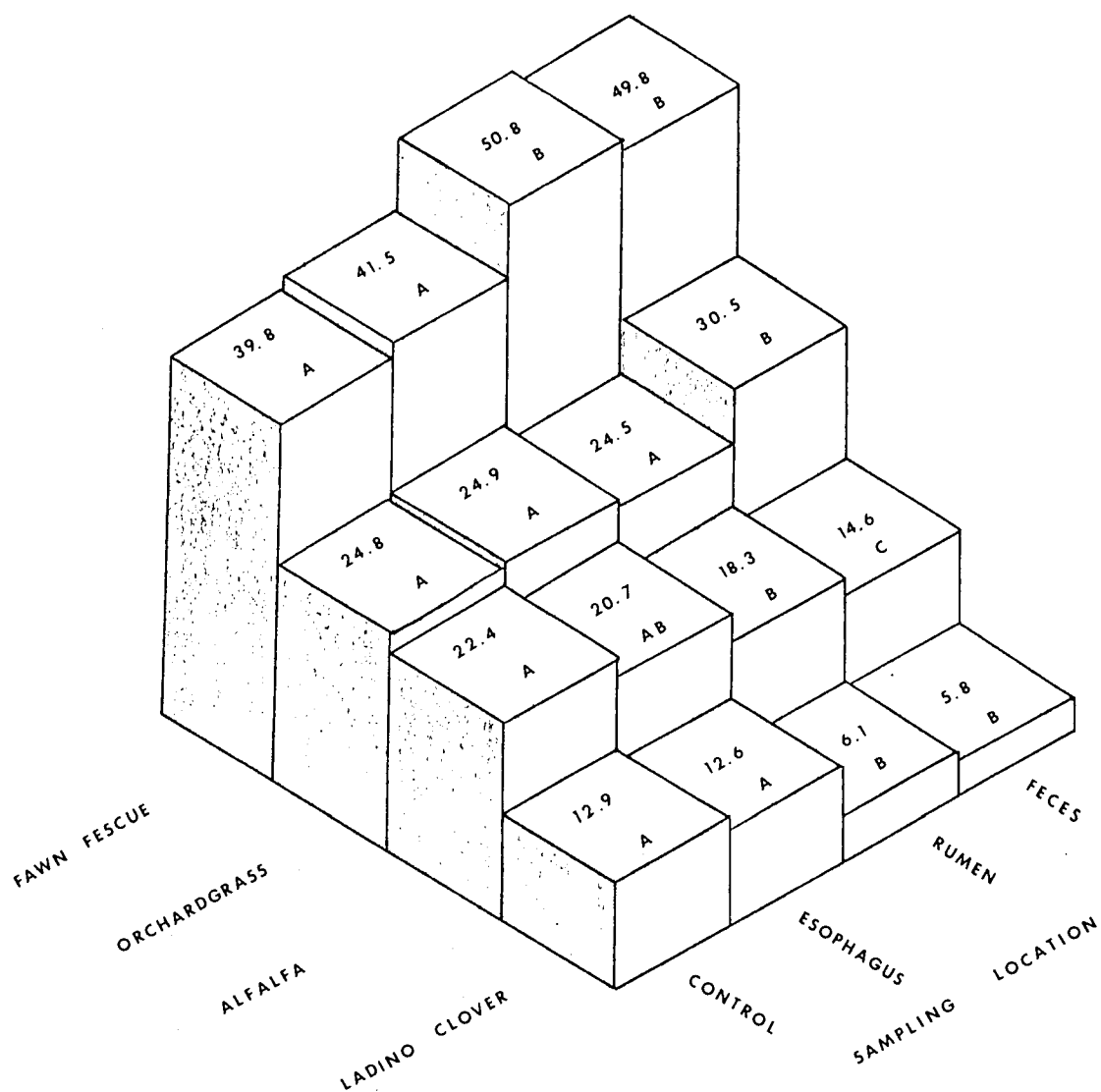
Figure 10. Percent dry weight composition of fawn fescue, orchard-grass, alfalfa, and ladino clover in each sampling location. Treatments with the same letters within plant species are not significantly different at the 95 percent confidence level.

no significant difference between the amount in the control and that
found in esophageal ingesta.

Ladino Clover

The amount of ladino clover in the control was significantly
higher than in rumen and fecal samples, but was not significantly
different than the amount contained in esophageal samples (Figure 10).
There was no significant difference between the amounts in fecal and
rumen samples.

The similarity between the botanical composition of the total
diet (individual species ignored) for the control and each sampling
location is shown in Table 2.

Table 2. Matrix indicating similarity between botanical composition
of the diet (species ignored) for the control and each
sampling location.

Sampling	Similarity index			
location	Control	Esophagus	Rumen	Feces
Control	100	98	89	80
Esophagus	98	100	90	82
Rumen	89	90	100	88
Feces	80	82	88	100

Based on the above summary, the esophageal fistula method
described the botanical composition of the control more accurately

than the other methods. The composition of rumen ingesta was more similar to that of the control than was fecal material.

There was a consistent disappearance of forbs as they passed through the digestive tract as indicated by lower percentages contained in the feces than in the rumen, and lower percentages contained in the rumen than in the esophagus (Figure 10).

The most likely explanation for the disappearance of forbs is that cell wall constituents were slowly eroded by digestion as the plant fragments passed through the digestive tract, rendering discernibility increasingly difficult. The fact that the composition of ladino clover and alfalfa in esophageal ingesta was slightly less than that of the control (Figure 10) suggests that these forbs were mechanically injured by mastication to the extent that they became slightly less discernible in this sampling location.

Rumen samples contained significantly less ladino clover and alfalfa than did the control (Figure 10). This relationship was consistent with the findings of Regal (1960), and may again be explained by differential digestibility of plant fragments.

The idea of cellular erosion of plant fragments due to differential digestibility among species is further substantiated by greater discernibility of ladino clover and alfalfa in the control than in the feces.

Since the diets were expressed in percentage composition, the percent of any one component in a sample was dependent on the proportion of the other components. Increasing underestimates of ladino clover and alfalfa from esophagus to rumen to feces resulted in increasing overestimates of orchardgrass and fawn fescue.

Early Summer Grazing Trial

The relative percent dry weight composition of every species occurring in the diet was calculated for each of the four sampling methods. The relative percent dry weight compositions of the total graminoid component and the total forb component were also determined.

Total Graminoids

Determination of the graminoid component by the ocular-estimate-by-plot method (OEBP) was significantly lower than by other methods. The total graminoid composition of esophageal samples was significantly lower than that of both rumen and fecal samples. There was no significant difference between rumen and fecal samples. The results can be summarized as follows[3]:

OEBP	Esophageal	Rumen	Fecal
35.6%	50.4%	67.4%	72.7%

[3]Treatment means are ranked from smallest to largest. Treatment means underscored with a common line are not significantly different at the 95% confidence level.

Total Forbs

The total forb component as determined by OEBP was significantly higher than for any other method. The amount of forbs in esophageal samples was significantly higher than in both rumen and fecal samples. Fecal samples and rumen samples did not contain significantly different amounts of forbs.

Fecal	Rumen	Esophageal	OEBP
26.8%	33.0%	49.4%	64.2%

Kentucky Bluegrass

Determination of Kentucky bluegrass by OEBP was significantly lower than by other methods. The amount identified in esophageal samples was significantly lower than that found in both rumen and fecal samples. The means for rumen and fecal samples were not significantly different.

OEBP	Esophageal	Rumen	Fecal
8.6%	23.7%	34.7%	36.4%

Timothy

Significant differences for timothy did not exist among rumen analysis, fecal analysis, and OEBP. The amount identified in esophageal samples was significantly lower than the amount identified in the other sampling locations.

Esophageal	Rumen	OEBP	Fecal
8.9%	13.5%	15.3%	15.8%

Prairie Junegrass

The amounts of prairie junegrass in rumen samples and fecal samples were not significantly different. However, the amount of this grass in esophageal ingesta samples was significantly higher than any other treatment. The amount of prairie junegrass in the diets as determined by OEBP was significantly lower than any other treatment.

OEBP	Rumen	Fecal	Esophageal
0.5%	1.8%	2.2%	3.5%

Smooth Brome

Significant differences did not exist between OEBP and rumen analysis. OEBP showed significantly lower amounts of smooth brome than esophageal and fecal samples. The amounts contained in esophageal, rumen, and fecal samples were not significantly different.

OEBP	Rumen	Esophageal	Fecal
0%	1.1%	1.6%	1.8%

California Danthonia

The amount of California danthonia was significantly lower for OEBP than for any other method. There were no significant

differences among amounts of this grass in rumen, esophageal and fecal samples.

OEBP	Rumen	Esophageal	Fecal
0.2%	1.2%	1.2%	1.6%

Western Needlegrass

The determination of western needlegrass by OEBP was significantly lower than by other methods. The composition of this grass in esophageal samples was not significantly different than in rumen samples but was significantly lower than the mean amount in feces. Significant differences did not exist between rumen and fecal samples.

OEBP	Esophageal	Rumen	Fecal
0%	1.0%	1.5%	1.7%

Soft Brome

The amount of soft brome in the diet as determined by OEBP was significantly lower than that identified in any other diet sample. The amount of this grass in esophageal samples was significantly higher than the amount consumed as determined by OEBP, but significantly lower than the amounts in rumen and fecal samples. Rumen and fecal samples were not significantly different.

OEBP	Esophageal	Rumen	Fecal
0%	0.7%	1.6%	1.7%

Idaho Fescue

Significant differences for Idaho fescue did not occur between OEBP and fecal samples. Both of these methods, however, had means which were significantly lower than esophageal and rumen samples. There were no significant differences between the amounts of esophageal and rumen samples.

OEBP	Fecal	Esophageal	Rumen
0%	t	0.1%	0.1%

Field Horsetail

Determination of field horsetail by OEBP was significantly lower than by fecal, esophageal, or rumen analysis. There were no significant differences among fecal, esophageal, and rumen samples.

OEBP	Fecal	Esophageal	Rumen
5.4%	12.9%	13.0%	13.8%

Beauty Cinquefoil

Significant differences for beauty cinquefoil did not occur between fecal and rumen samples. Both of these methods, however, were significantly lower than OEBP and esophageal fistula. There was no significant difference between the amount of this species in esophageal samples and OEBP.

Fecal	Rumen	Esophageal	OEBP
5.9%	8.0%	20.5%	30.5%

Oregon Checkermallow

There were no significant differences among fecal, rumen, and esophageal samples. Fecal and rumen samples contained significantly less of this species than the amount as determined by OEBP. There was no significant difference between the amount of Oregon checkermallow in esophageal ingesta and that as determined by OEBP.

Fecal	Rumen	Esophageal	OEBP
1.2%	2.0%	5.5%	10.0%

Yellow Salsify

The OEBP of yellow salsify was significantly lower than the amount identified in any other sampling location. Significant differences did not occur among fecal, esophageal, and rumen samples.

OEBP	Fecal	Esophageal	Rumen
2.7%	6.8%	7.9%	8.6%

Common Dandelion

While the amounts of common dandelion identified in fecal and rumen samples were not significantly different, both of these sampling locations contained significantly lower amounts than the OEBP and the amount identified in esophageal samples.

Fecal	Rumen	OEBP	Esophageal
0%	t	1.0%	1.3%

Sticky Geranium

There was no sticky geranium in the diets as determined by OEBP, fecal analysis, and rumen analysis. The amount contained in esophageal samples was significantly higher than that identified by other methods.

OEBP	Fecal	Rumen	Esophageal
0%	0%	0%	t

Missouri Goldenrod

There were no significant differences among the amounts of Missouri goldenrod contained in fecal, rumen, and esophageal samples. The OEBP of this species was significantly higher than the amount contained in any other sampling location.

Fecal	Rumen	Esophageal	OEBP
0%	0%	t	1.4%

Orange Arnica

Fecal, rumen, and esophageal samples did not contain significantly different amounts of orange arnica. The OEBP was significantly higher than the amounts in the diets as determined by other methods.

Fecal	Rumen	Esophageal	OEBP
0%	t	t	1.0%

Pale Agoseris

The amounts of pale agoseris in fecal, rumen, and esophageal samples were not significantly different, but were significantly lower than OEBP.

Fecal	Rumen	Esophageal	OEBP
0%	0%	t	3.7%

Of the 13 graminoids occurring on the study area (Appendix C), a total of 11 were identified in diet samples. All of these were found in diet samples collected from the esophagus and rumen (Table 3). OEBP failed to show the presence of four species of graminoids. Only one species of grass was not found in fecal samples. There were significant differences among the mean values of the sampling techniques for eight of the graminoids. The graminoids which showed no significant differences among treatment means are listed in Table 4, together with the corresponding mean dry weight composition as determined by each technique.

Of the 34 species of forbs occurring on the study area (Appendix C), a total of 20 were identified in diet samples. All of these were found in esophageal ingesta samples (Table 3). Sixteen species of forbs were found in rumen ingesta, five species of forbs were

Table 3. Presence (+) and absence (0) of species in each sampling location for the early summer grazing trial.

Species	Treatment			
	Esophageal	Rumen	Fecal	OEBP[b]
Graminoids				
Kentucky bluegrass	+	+	+	+
Timothy	+	+	+	+
Baltic rush	+	+	+	+
Sedge[a]	+	+	+	+
Prairie junegrass	+	+	+	+
Smooth brome	+	+	+	0
Western needlegrass	+	+	+	0
California danthonia	+	+	+	+
Soft brome	+	+	+	0
Canada bluegrass	+	+	0	+
Idaho fescue	+	+	+	0
Forbs				
Field horsetail	+	+	+	+
Beauty cinquefoil	+	+	+	+
Oregon checkermallow	+	+	+	+
Yellow salsify	+	+	+	+
Common dandelion	+	+	+	+
Gland cinquefoil	+	+	0	+
Prairiesmoke avens	+	0	0	0
Rose pussytoes	+	+	0	+
Hook violet	+	+	0	+
Sticky geranium	+	0	0	0
Yarrow	+	+	0	+
Blueleaf strawberry	+	+	0	+

(Continued on next page)

Table 3. (Continued)

Species	Treatment			
	Esophageal	Rumen	Fecal	OEBP[b]
American vetch	+	+	0	+
Missouri goldenrod	+	0	0	+
Rockymountain iris	+	+	0	+
Shaggy fleabane	+	+	0	0
Orange arnica	+	+	0	+
Autumn willowweed	+	+	0	+
Sheep sorrel	+	+	0	+
Pale agoseris	+	0	0	+

[a]Sedges not identified to species.

[b]Ocular-estimate-by-plot.

Table 4. Species showing no significant differences among treatment means. Values listed are dry weight compositions (percent) of plant fragments identified in diets by each technique.

Species	Esophageal	Rumen	Fecal	OEBP[c]
Graminoids				
Baltic rush	0.93	1.5	1.8	1.8
Sedges[a]	8.8	9.9	9.6	8.9
Canada bluegrass	0.21	0.28	0	0.24
Forbs				
Gland cinquefoil	t[b]	t	0	t
Rose pussytoes	t	t	0	t
Hook violet	t	t	0	t
Yarrow	0.1	0.1	0	1.3
Blueleaf strawberry	t	t	0	t
American vetch	0.2	t	0	6.5
Rockymountain iris	0.1	t	0	0.6
Shaggy fleabane	t	t	0	0
Autumn willowweed	t	t	0	0.1
Sheep sorrel	t	0.1	0	0.2
Prairiesmoke avens	t	t	0	t

[a]Sedges not identified to species.

[b]Less than 0.1%.

[c]Ocular-estimate-by-plot.

identified in fecal samples, and 17 species of forbs were determined to be dietary components by the ocular-estimate-by-plot method.

There were significant differences among the mean values of the sampling methods for nine of the forb species occurring in the diet. Those forbs which showed no significant differences among treatment means are listed in Table 4, together with the corresponding mean dry weight composition as determined by each technique.

Late Summer Grazing Trial

The relative percent dry weight composition of every species in the diet was calculated for each of the four sampling techniques. The relative percent dry weight compositions of the total graminoid component and the total forb component were also determined.

Total Graminoids

Significant differences did not occur between the total amount of graminoids in the feces and rumen samples. The total graminoid content of esophageal samples was significantly lower than that of rumen and fecal samples. The ocular-estimate-by-plot method (OEBP) showed significantly lower amounts of graminoids than any other method.

OEBP	Esophageal	Rumen	Fecal
29.5%	50.5%	67.1%	70.5%

Total Forbs

The forb component of esophageal samples was not significantly different than the OEBP. Both of these methods, however, demonstrated significantly higher amounts of forbs than those identified in fecal and rumen samples. The forb content of the feces was not significantly different than that of rumen ingesta.

Fecal	Rumen	Esophageal	OEBP
28.6%	32.7%	49.8%	60.9%

Kentucky Bluegrass

The OEBP for Kentucky bluegrass was significantly lower than any other method. The amount of this species in esophageal samples was significantly lower than that in rumen and fecal samples. Rumen samples and fecal samples did not contain significantly different amounts of the grass.

OEBP	Esophageal	Rumen	Fecal
13.2%	27.0%	37.9%	41.9%

Timothy

The amount of timothy in the diet as determined by OEBP was significantly less than by other methods. Esophageal samples contained significantly less than rumen and fecal samples. Significant differences did not occur between rumen and fecal samples.

OEBP	Esophageal	Rumen	Fecal
t	12.5%	17.4%	17.8%

Smooth Brome

Rumen analysis and OEBP did not demonstrate significant differences of smooth brome. Rumen samples and esophageal samples did not contain significantly different amounts. The amount identified in fecal samples was significantly higher than determined by all other techniques.

OEBP	Rumen	Esophageal	Fecal
0	0.19%	0.26%	0.5%

California Danthonia

There were no significant differences among the amounts of California danthonia contained in fecal, esophageal and rumen samples. Each of these sampling locations, however, contained significantly higher amounts than OEBP.

OEBP	Fecal	Esophageal	Rumen
0	0.9%	1.0%	1.0%

Soft Brome

There were significant differences among all methods. The greatest amount of soft brome was identified in fecal samples, followed by rumen samples, esophageal samples, and finally OEBP.

OEBP	Esophageal	Rumen	Fecal
0	0.3%	0.9%	1.2%

Oregon Checkermallow

The amount of Oregon checkermallow identified in fecal samples was not significantly different than that contained in rumen ingesta. The amount contained in fecal samples, however, was significantly lower than the OEBP and the amount contained in esophageal samples. The amount of Oregon checkermallow identified in rumen ingesta was not significantly different than the OEBP, but was significantly lower than that recorded in esophageal samples. There was no significant difference between the OEBP and esophageal fistula methods.

Fecal	Rumen	OEBP	Esophageal
2.3%	3.2%	6.3%	7.8%

Common Dandelion

Significant differences for common dandelion did not occur among fecal analysis, rumen analysis, and OEBP. The amount contained in esophageal samples was significantly higher than that as determined by all other methods.

Fecal	OEBP	Rumen	Esophageal
0	t	0.2%	2.2%

Velvet Lupine

Amounts of velvet lupine contained in fecal, rumen, and esophageal samples were not significantly different. The OEBP was significantly higher than the amount contained in all other sampling locations.

Fecal	Rumen	Esophageal	OEBP
0.2%	0.7%	2.2%	19.6%

A total of ten different species of graminoids were identified in diet samples (Table 5). All of these were found in esophageal and rumen ingesta. Fecal samples contained nine different graminoid species, and ocular estimates of utilization showed that six species of graminoids were present in the diet. Of the ten species of graminoids consumed, five had treatment means which were not significantly different (Table 6).

A total of 15 different species of forbs were present in diet samples (Table 5). All of these were found in esophageal samples, nine were found in rumen and six were identified in fecal material. Fourteen species of forbs were identified by the ocular-estimate-by-plot method as being present in the diet. Twelve of the 15 forb species present in the diet had treatment means which were not significantly different (Table 6).

Table 5. Presence (+) and absence (0) of species in each sampling location for the late summer grazing trial.

Species	Treatment			
	Esophageal	Rumen	Fecal	OEBP[b]
Graminoids				
Kentucky bluegrass	+	+	+	+
Timothy	+	+	+	+
Baltic rush	+	+	+	+
Sedges[a]	+	+	+	+
Prairie junegrass	+	+	+	+
Smooth brome	+	+	+	0
Western needlegrass	+	+	+	+
California danthonia	+	+	+	0
Soft brome	+	+	+	0
Idaho fescue	+	+	0	0
Forbs				
Field horsetail	+	+	+	+
Beauty cinquefoil	+	+	+	+
Oregon checkermallow	+	+	+	+
Missouri goldenrod	+	+	+	+
American vetch	+	0	0	0
Common dandelion	+	+	0	+
Prairiesmoke avens	+	0	0	+
Rockymountain iris	+	0	0	+
Sheep sorrel	+	0	0	+
Rose pussytoes	+	+	0	+
Yellow salsify	+	+	+	+
Yarrow	+	0	0	+
Velvet lupine	+	+	+	+
Blueleaf strawberry	+	0	0	+
Pale agoseris	+	+	0	+

[a]Sedges not identified to species.

[b]Ocular-estimate-by-plot.

Table 6. Species of the late summer trial with no significant differences among treatment means. Values are dry weight compositions (percent) of plant fragments identified by each technique.

Species	Treatment			
	Esophageal	Rumen	Fecal	OEBP[c]
Graminoids				
Baltic rush	0.57	0.81	0.70	1.6
Sedges[a]	3.7	4.8	4.7	9.1
Prairie junegrass	4.2	2.9	2.6	4.4
Western needlegrass	0.84	1.3	1.3	0.90
Idaho fescue	t[b]	t	0	0
Forbs				
Field horsetail	8.3	12.3	11.6	7.0
Beauty cinquefoil	22.7	10.7	9.4	20.9
Missouri goldenrod	0.13	t	1.3	0.13
American vetch	t	0	0	0
Prairiesmoke avens	t	0	0	0.1
Rockymountain iris	t	0	0	t
Sheep sorrel	t	0	0	0.2
Rose pussytoes	0.3	0.1	0	t
Yellow salsify	5.5	5.7	3.6	5.5
Yarrow	t	0	0	0.3
Blueleaf strawberry	0.1	0	0	0.9
Pale agoseris	0.3	t	0	t

[a]Sedges not identified to species.

[b]Less than 0.1%.

[c]Ocular-estimate-by-plot.

Evaluation of Methods

Ocular-Estimate-by-Plot Method

The ocular-estimate-by-plot method failed to show the presence of several species of grasses and forbs. There are two different explanations of this. Diminutive annuals (e.g. soft brome) possess weak rooting systems. It is conceivable that the roots, stems, and leaves of such plants were entirely consumed by sheep, thereby leaving no standing residue as evidence that the plant had ever been utilized. Laycock et al. (1972) observed such "invisible" utilization of mountain knotweed (Polygonum montanum).

The second reason for the failure of the ocular-estimate-by-plot method to identify certain species in the diet is probably due to sampling error. Many species occurred on the study area in relatively low frequencies (Appendix F). The ten plots per pasture used in this study gave reasonably precise estimates of dry weight production; the half-confidence interval of total herbage production in the early summer trial was 14% of the mean at the 95% confidence level. However, it is probable that species with relatively low frequencies of occurrence on the study area required larger sampling sizes to decrease the amount of variation among means.

California danthonia, as an example, had a mean frequency of occurrence of 19% on the study area (Appendix F) and yielded

production estimates witha half-confidence interval of 68% of the mean at the 95% confidence level in the early summer trial. Thus, it may be assumed that inadequate sampling sizes for less abundant species probably resulted in erroneous dietary compositions.

This conclusion is further substantiated by one other confounding event. Percentage utilization of species in the late summer trial was derived by subtracting the estimated utilization of species of the early summer trial from the combined percent utilization of both trials. In some species, no value could be assigned to utilization for the late summer trial because the combined utilization was less than the utilization of species in the early trial. It is believed that regrowth of vegetation between trials was not great enough to account for this entire phenomenon, and that some portion of it must be due to inadequate sampling size.

Due to these errors, a more clear picture of the relative values of the ocular-estimate-by-plot, esophageal fistula, stomach content analysis, and fecal analysis methods may be had by focusing attention only on certain "target" species. Plants will be designated as "target" species if they meet either of the following criteria:

1. Major dietary component of the early summer trial showing a reasonably high frequency of occurrence on the study area; or

2. Major dietary component of the late summer trial showing a reasonably high frequency of occurrence on the study area, and

utilized less in the early summer trial than in the combined

estimates of the early and late trials.

Plants identified as "target" species are Kentucky bluegrass,

timothy, field horsetail, beauty cinquefoil, Oregon checkermallow,

yellow salsify, and velvet lupine.

Of the "target" graminoids, timothy was the only species for

which the ocular-estimate-by-plot method did not identify significantly

less amounts in the diet than other methods. The probable explanation

of this is that the identification of timothy and Kentucky bluegrass was

occasionally reversed in rumen, esophageal, and fecal samples due to

cuticular similarities between species. When the means of these two

species in each sampling location were combined, Duncan's new

multiple-range test (Steel and Torrie, 1960) provided the following

results:

OEBP	Esophageal	Rumen	Fecal
11.9%	16.4%	24.2%	26.1%

Thus, the dietary composition of "target" grasses based on the

ocular-estimate-by-plot method was lower than that as determined by

other techniques. This trend was partially substantiated by Laycock

et al. (1972) who found that the composition of grasses in diets as

determined by the ocular-estimate-by-plot method was less than by

the esophageal fistula method.

The ocular-estimate-by-plot method tended to yield higher values for "target" forbs than the other techniques. For two of these species (beauty cinquefoil and Oregon checkermallow) utilization estimates were slightly, but not significantly higher than percent composition as determined by the esophageal fistula method. This is consistent with the observations of Laycock et al. (1972).

Species of notable exception to this trend were field horsetail, yellow salsify, and velvet lupine. Field horsetail and yellow salsify were determined to be significantly greater portions of the diet by esophageal, fecal, and rumen analysis than by the ocular-estimate-by-plot methods. These species were two of the most easily discernible forbs in the diet samples. It may be reasonable to assume that field horsetail and yellow salsify were overestimated at the expense of species which produced tissue residues that were less discernible or less persistent.

The ocular-estimate-by-plot method showed significantly greater amounts of velvet lupine than in esophageal, rumen, or fecal samples. This situation was due to the poor discernibility of the plant fragments in the diet samples. This forb was utilized only in the late summer trial, and it was observed that only the legumes were consumed. While legumes from this plant were observed in gross esophageal and rumen ingesta samples, fragments of this species could be identified only infrequently on microscope slides.

Stomach Content Analysis

The gross composition of the diet as determined by rumen analysis tended to be higher in graminoids and lower in forbs for both trials than that as determined by the ocular-estimate-by-plot method and the esophageal fistula method (Figures 11 and 12). However, rumen ingesta generally contained fewer graminoids and more forbs than fecal material. Ignoring species, there was greater similarity between diets as determined by rumen analysis and fecal analysis than between rumen analysis and any other technique.

The total forb component of rumen ingesta was significantly lower than that of esophageal ingesta for both trials. It was observed that while the discernibility of graminoids was about the same in both rumen and esophageal ingesta, fragments of forb species were more difficult to recognize in rumen samples. The likelihood is that cellular erosion due to digestion was greater for forbs in the rumen, thus decreasing discernibility of these plant fragments in this sampling location.

There is a second explanation for lower amounts of forbs in rumen samples than in esophageal samples. Norris (1943) noted that succulent forages passed through the stomach more rapidly than coarse, fibrous portions of the diet. Assuming that such "throughput" time was more rapid for forbs than for grasses in the present study,
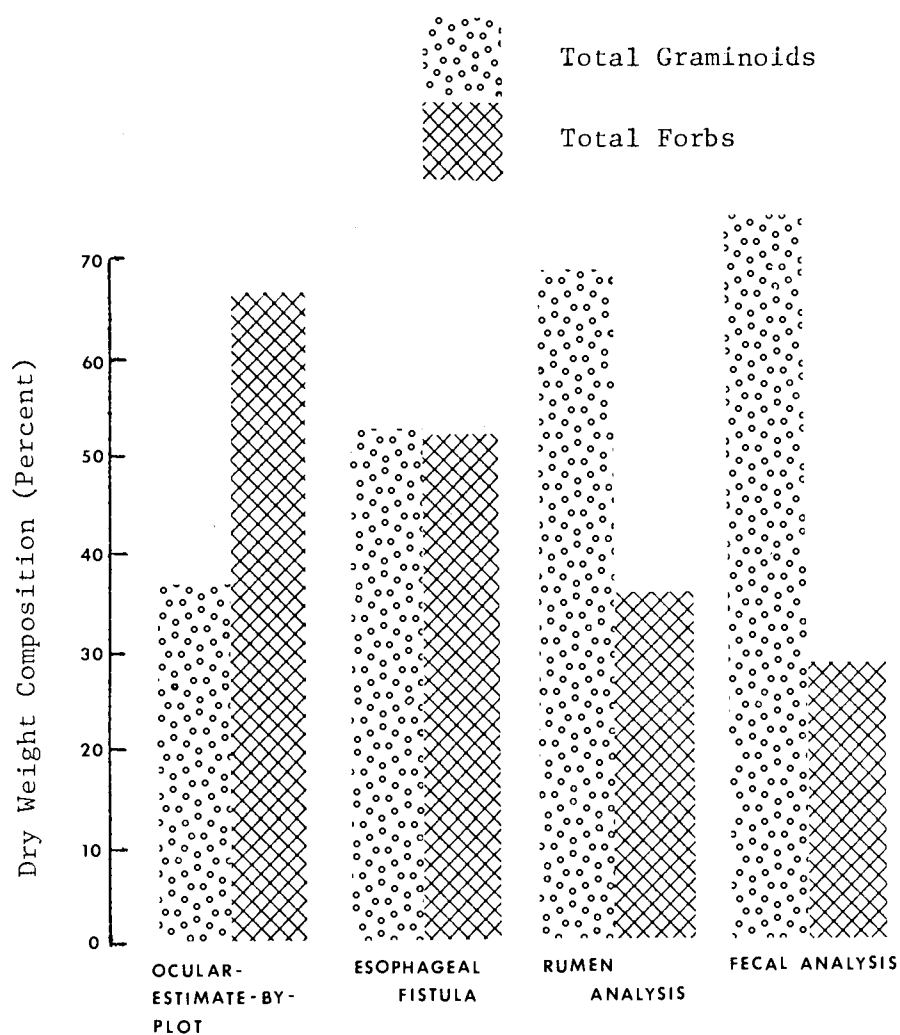
Figure 11. Dry weight composition (percent) of the total forb and total graminoid components of the diet for the early summer trial as determined by four methods.
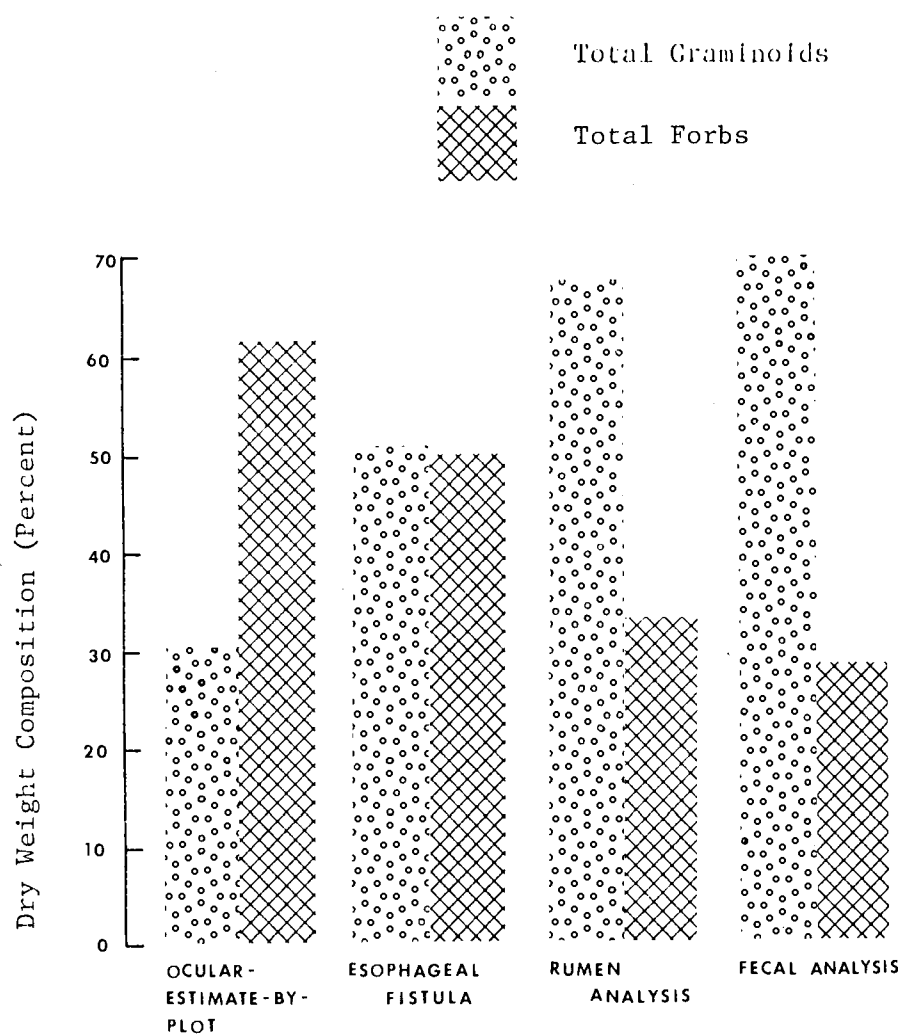
Figure 12. Dry weight composition (percent) of the total
forb and total graminoid components of the diet
for the late summer trial as determined by four
methods.

rumen analysis would tend to overestimate the abundance of grami-
noids and underestimate the abundance of forbs.

Esophageal Fistula Method

It is tempting to regard the esophageal fistula method as the
standard against which the other methods may be compared. Micro-
scopic analysis of esophageal ingesta was the single most accurate
method of estimating diets under the constraints of the feeding trial.
It was observed that the discernibility of plant fragments in esophageal
ingesta was greater than that in rumen or fecal samples. Persistence
of plant fragments in esophageal ingesta was greater than that of any
other sampling location since the effects of cellular erosion due to
digestion were probably least. Ingesta collected from the esophagus
was an actual portion of the diet, and as such was not subject to the
inherent sampling errors of the ocular-estimate-by-plot method.

However, there are at least two reasons for regarding the
esophageal fistula method with some suspicion. The matter of
incomplete recovery of fistula samples has previously been discussed.
If (as suggested by Lesperance et al., 1960a) only less fibrous por-
tions of the boluses are collected, it may be reasonable to assume
that the composition of the diet would tend to be higher in forbs and
lower in grasses for at least the early summer trial. However, the
graminoid component was just slightly higher than the forb component

(Figure 11). In the late summer trial there was virtually no difference between the total graminoid composition and the total forb composition of the diet as determined by the esophageal fistula technique (Figure 12).

There is a second opportunity for error when the esophageal fistula method is used to estimate the composition of diets. Plant fragments found in esophageal ingesta represent the diet of the animal for only that length of time during which the fistula sample is being collected. Rumen and fecal samples, however, are composed of plants which have been eaten at least throughout the entire day. If different species or different amounts of plants are consumed during the time when the fistula is open than for other times of the day, an erroneous composition of the diet will result.

Fecal Analysis

Microscopic examination of fecal material has become one of the most popular methods of determining the food habits of large hetbivores. The technique is advantageous because it allows the sampling of numerous individuals over fairly large areas; it offers practically unlimited sampling; it does not interfere with the normal behavior of animals; and it can be used when several types of herbi-vores are utilizing the same range.

However, several limitations of the technique have become apparent in this study. Microscopic examination of fecal samples showed a higher composition of total graminoids and lower composition of total forbs in the diet for both grazing trials than any other method (Figures 11 and 12). Diets as determined by fecal analysis were not as diverse as those determined by the other methods since several minor forbs were not found in feces (Tables 3 and 5).

These observations are consistent with the findings of other workers (Vavra et al., 1970; Korfhage, 1974) and may be explained partially on the basis of differential digestibility. It is conceivable that certain species of forbs are entirely digested leaving no residue in the feces. In other instances, plant fragments were present but were so transparent that cellular structure was not easily discernible. This was particularly true with Oregon checkermallow. Identification of this species in fecal material was based more often on the unique characteristics of its stellate trichomes than on cellular structure. The accuracy of fecal analysis could probably be enhanced by the determination of digestibility coefficients of various plant species in different phenological stages, and for different animal species.

Unlike rumen and esophageal ingesta, fecal samples in this study were not ground in a Wiley mill prior to the preparation of microscope slides. It was observed by initial experimentation that ground fecal material resulted in slides with fewer identifiable plant

fragments than unground fecal material. This observation was also made by Slater and Jones (1971) who found less clover in ground fecal material than in unground portions of the same sample. It is possible that by not grinding samples, plant fragments of varying sizes resulted, thereby overestimating the abundance of some species.

Similarity of Methods

The composition of "target" grasses in the diet as determined by the ocular-estimate-by-plot method demonstrated less similarity to other methods than the other methods did among themselves (Table 7).

Table 7. Matrix indicating similarity between composition of combined "target" grasses in the diet for both grazing trials as determined by four methods of dietary analysis.

Method	Similarity index			
	OEBP[a]	Esophageal	Rumen	Fecal
OEBP[a]	100	67	52	49
Esophageal	67	100	82	78
Rumen	52	82	100	96
Fecal	49	78	96	100

[a]Ocular-estimate-by-plot

The composition of "target" forbs in the diet as determined by the ocular-estimate-by-plot method compared favorably with the esophageal fistula method, but not with rumen and fecal analysis (Table 8).

Table 8. Matrix indicating similarity between composition of combined "target" forbs in the diet for both grazing trials as determined by four methods of dietary analysis.

Method	Similarity index			
	OEBP[a]	Esophageal	Rumen	Fecal
OEBP[a]	100	92	67	66
Esophageal	92	100	74	72
Rumen	67	74	100	99
Fecal	66	72	99	100

[a]Ocular-estimate-by-plot

The true similarity of the ocular-estimate-by-plot method to the other methods may have been masked by sampling error. In this study it was assumed that a one-to-one ratio existed between relative density of plant fragments identified in microscope fields and dry weight composition of these fragments (Sparks and Malechek, 1968). If this relationship was invalid in this study, diets as determined by the ocular-estimate-by-plot method would not be comparable to diets as determined by other methods. However, since a common base was used in determining the dry weight compositions of diets by the esophageal fistula, stomach content analysis, and fecal analysis methods, it may be reasonable to assume that the similarity indices among these methods are correct.

Precision of Methods

An attempt was made to evaluate the precision of each method

on the basis of the coefficient of variability (Steel and Torrie, 1960).

However, no single method demonstrated consistent reliability over

both grazing trials and both vegetation types (grasses and forbs).

Rumen analysis and fecal analysis tended to be more precise in their

estimates of dietary composition of grasses and forbs than other

methods (Table 9). This was probably a reflection of the lack of

species diversity in these two sampling locations. Species with

apparent low resistence to cellular erosion were not frequently

identified in rumen or fecal samples. Thus, fewer species occurred

in these sampling locations with greater regularity.

The precision of diets as determined by the ocular-estimate-

by-plot method would probably inncrease with a larger sampling size

than was used in this study. In most instances this would be possible

since the time required to sample by this method was less than the

other methods.

Application of Methods

Knowledge of the absolute amounts of plants consumed by large

herbivores may be important to researchers who wish to compare

food habits within or among animal species. However, wildland

managers are often more interested in the relative importance, rather

than the absolute amounts of plant species occurring in the diets of

large herbivores.

Table 9. Mean composition (percent dry weight) of all graminoids and all forbs in each pasture of both grazing trials together with percent coefficient of variability (cov).

Methods	Replications (pastures)							cov (%)
	1	2	3	4	5	6	7	

Early summer trial

Graminoids
Esophageal fistula	51.5	60.3	34.8	60.0	52.0	59.3	35.2	20
Rumen analysis	75.3	62.6	43.0	71.9	64.7	78.0	76.2	17
Fecal analysis	79.9	65.7	58.3	75.4	69.0	82.6	77.8	11
Ocular-estimate-by-plot	40.6	56.2	13.5	12.7	34.9	42.9	48.5	43

Forbs
Esophageal fistula	48.4	39.6	65.2	39.8	47.8	40.4	64.6	20
Rumen analysis	24.4	37.2	57.0	27.8	35.8	21.6	27.8	33
Fecal analysis	20.8	31.2	41.8	24.6	30.8	17.2	21.8	29
Ocular-estimate-by-plot	58.8	43.6	86.4	87.2	65.0	57.0	51.4	24

Late summer trial

Graminoids
Esophageal fistula	58.4	48.9	41.4	48.7	55.0			10
Rumen analysis	69.3	61.0	68.1	66.7	70.4			5
Fecal analysis	74.8	67.3	63.7	73.3	73.4			6
Ocular-estimate-by-plot	52.2	25.6	14.5	16.2	39.8			48

Forbs
Esophageal fistula	41.5	51.5	59.1	51.2	45.8			12
Rumen analysis	30.6	38.4	32.3	32.7	29.9			9
Fecal analysis	25.4	33.9	30.5	26.4	26.9			11
Ocular-estimate-by-plot	47.7	72.5	84.8	79.5	20.4			39

Relative preference indices were computed for those plant species of each grazing trial which demonstrated at least 1% composition of the diets by at least one of the methods tested. These values were ranked in order of magnitude to compare the ability of each method in evaluating the relative importance of plant species occurring in the diets (Tables 10 and 11).

The esophageal fistula, stomach content analysis, and fecal analysis methods were comparable in their rankings of relative preference values with only minor variations being demonstrated. These were largely unremarkable with the possible exception of fecal analysis in the early summer trial (Table 10). Five species of forbs were not identified in fecal material, and thus could not be assigned relative preference indices. Assuming that differential digestibility of plant tissues yields lower composition of certain species in fecal material, it is reasonable to suggest that the relative preference indices of these species may be underestimated by fecal analysis in the succulent phenological stages of the plants.

The ocular-estimate-by-plot method was not as comparable to the other methods in its ranking of relative preference values of species as the other methods were among themselves. Smooth brome, as an example, was the most highly preferred grass during the early summer trial as determined by the esophageal fistula, stomach content analysis, and fecal analysis methods (Table 10). This grass, however,

Table 10. A comparison of the relative indices (RPI) of species occurring in the diets of sheep for the early summer grazing trial as determined by four methods of dietary analysis.

Ocular-estimate-by-plot		Esophageal fistula		Stomach content analysis		Fecal analysis	
Species	RPI	Species	RPI	Species	RPI	Species	RPI
Graminoids							
Timothy	4.0	Smooth brome	17.7	Smooth brome	12.2	Smooth brome	20.0
Baltic rush	2.3	Prairie junegrass	3.3	Timothy	3.6	Timothy	4.1
Sedges	1.3	Kentucky bluegrass	2.5	Kentucky bluegrass	3.6	Kentucky bluegrass	3.8
Kentucky bluegrass	0.9	California danthonia	2.5	California danthonia	2.5	California danthonia	3.4
Prairie junegrass	0.5	Timothy	2.3	Western needlegrass	2.1	Western needlegrass	2.4
California danthonia	0.4	Western needlegrass	1.4	Baltic rush	1.8	Baltic rush	2.3
Soft brome	0	Baltic rush	1.1	Soft brome	1.8	Prairie junegrass	2.1
Western needlegrass	0	Sedges	1.0	Prairie junegrass	1.7	Soft brome	1.8
Smooth brome	0	Soft brome	0.8	Sedges	1.1	Sedges	1.0
Forbs							
Field horsetail	9.0	Field horsetail	21.6	Field horsetail	23.0	Field horsetail	21.5
Yellow salsify	6.8	Yellow salsify	19.8	Yellow salsify	21.5	Yellow salsify	17.0
Hook violet	3.3	Common dandelion	1.8	Oregon checkermallow	0.6	Oregon checkermallow	0.4
Oregon checkermallow	3.0	Oregon checkermallow	1.6	Beauty cinquefoil	0.3	Beauty cinquefoil	0.2
Pale agoseris	2.3	Beauty cinquefoil	0.6	Hook violet	t	Hook violet	0
Orange arnica	1.5	Hook violet	0.1	Common dandelion	t	Common dandelion	0
Common dandelion	1.4	Orange arnica	t	Orange arnica	t	Orange arnica	0
Beauty cinquefoil	0.9	Pale agoseris	t	Pale agoseris	0	Pale agoseris	0
Missouri goldenrod	0.5	Missouri goldenrod	t	Missouri gondenrod	0	Missouri goldenrod	0

a Trace. Less than 0.1.

Table 11. A comparison of the relative preference indices (RPI) of species occurring in the diets of sheep for the late summer grazing trial as determined by four methods of dietary analysis.

Ocular-estimate-by-plot		Esophageal fistula		Stomach content analysis		Fecal analysis	
Species	RPI	Species	RPI	Species	RPI	Species	RPI
Graminoids							
Prairie junegrass	5.7	Prairie junegrass	5.4	Kentucky bluegrass	7.2	Kentucky bluegrass	7.9
Kentucky bluegrass	2.5	Kentucky bluegrass	5.1	Prairie junegrass	3.8	Soft brome	4.4
Baltic rush	2.1	California danthonia	2.4	Soft brome	3.3	Prairie junegrass	3.4
Sedges	0.9	Timothy	1.9	Western needlegrass	2.8	Timothy	2.8
Western needlegrass	0.2	Western needlegrass	1.8	Timothy	2.7	Western needlegrass	2.3
Timothy	t[a]	Soft brome	1.1	California danthonia	2.4	California danthonia	2.4
California danthonia	0	Baltic rush	0.8	Baltic rush	1.0	Baltic rush	0.9
Soft brome	0	Sedges	0.3	Sedges	0.5	Sedges	0.4
Forbs							
Yellow salsify	7.8	Yellow salsify	7.9	Field horsetail	10.7	Field horsetail	10.0
Field horsetail	6.1	Field horsetail	7.2	Yellow salsify	8.1	Yellow salsify	5.1
Oregon checkermallow	2.1	Common dandelion	3.0	Oregon checkermallow	1.0	Oregon checkermallow	0.8
Beauty cinquefoil	0.9	Oregon checkermallow	2.6	Beauty cinquefoil	0.4	Beauty cinquefoil	0.4
Velvet lupine	0.7	Beauty cinquefoil	0.9	Common dandelion	0.3	Velvet lupine	t
Common dandelion	t	Velvet lupine	t	Velvet lupine	t	Common dandelion	0

[a]Trace. Less than 0.1.

was not observed to have been utilized and therefore resulted in a relative preference index of zero for the ocular-estimate-by-plot method. The failure of this method to identify smooth brome as a dietary component was probably due to the sampling error previously discussed, and exemplifies the hazards of undersampling. Smooth brome exhibited a mean frequency of occurrence of only 1% on the study area (Appendix F) but was the single most important grass in the diets during the early summer trial.

SUMMARY AND CONCLUSIONS

The objectives of this study were to determine the diets of sheep grazing a common range in early summer (late June) and late summer (late August) by the esophageal fistula, stomach content analysis, fecal analysis, and ocular-estimate-by-plot methods; compare the diet compositions among methods; determine the diets of sheep fed a hand-composited diet of known composition using the esophageal fistula, stomach content analysis, and fecal analysis methods; and determine the accuracy and relative values of the esophageal fistula, stomach content analysis, and fecal analysis methods by comparing the composition of the diet as determined by each method to that as determined by each of the other two methods, and to the known composition of the hand-composited diet.

Rangeland grazing trials were conducted on a beauty cinquefoil/ velvet lupine - Kentucky bluegrass/timothy community of the Eastern Oregon Agricultural Research Center Hall Ranch, 12 miles southeast of Union, Oregon. Microscope slide mounts were made of plant fragments collected from the esophagus, rumen, and feces of bi-fistulated (esophagus and rumen) sheep. Ocular estimates of forage utilization were made concurrently. Data were converted to percent composition on a dry weight basis for comparisons.

Significant differences (p < .05) in percent diet composition among methods occurred for 18 of the 31 plant species consumed in the

early summer trial, and for 17 of the 31 plant species consumed in the late summer trial.

The ocular-estimate-by-plot method resulted in higher mean values for the composition of forbs and lower mean values for the composition of graminoids than any other method. Ocular estimates of utilization failed to show the presence of several species of grass and forbs. Part of this was attributed to sampling error, and part was due to "invisible" utilization. Dietary composition as determined by the ocular-estimate-by-plot method was more similar to the esophageal fistula method than any other technique.

Composition of the diets based on the esophageal fistula method tended to be lower in grasses and higher in forbs than as determined by rumen or fecal analysis, but higher in grasses and lower in forbs than the ocular-estimate-by-plot method. Discernibility of plant fragments was greater in esophageal ingesta than in rumen or fecal samples. There was greater similiarity between diets as determined by the esophageal fistula method and the ocular-estimate-by-plot method than between the esophageal fistula method and any other technique.

The gross composition of the diet as determined by rumen analysis tended to be higher in graminoids and lower in forbs for both trials than that as determined by the ocular-estimate-by-plot method and the esophageal fistula method. Rumen ingesta generally contained

fewer graminoids and more forbs than fecal material. There was greater similarity between diets as determined by rumen analysis and diets as determined by fecal analysis than between rumen analysis and any other method.

Microscopic examination of fecal samples showed a higher composition of graminoids and lower composition of forbs in the diet than other methods. Diets as determined by fecal analysis were not as diverse as those determined by the other methods. In most instances, the composition of fecal samples was more comparable to that of the rumen samples than the composition as determined by any other method.

In the second study phase, ten bi-fistulated sheep were fed a hand-composited diet of ladino clover, alfalfa, orchardgrass, and fawn fescue. Diets as determined by the esophageal fistula, rumen analysis, and fecal analysis methods were compared to one another and to the original hand-composited feed (control).

The esophageal fistula method described the composition of the original feed more accurately than the other methods. The esophageal fistula method demonstrated greater similarity to rumen analysis than to fecal analysis. Discernibility of plant fragments was greater in esophageal ingesta than rumen or fecal samples.

Rumen samples contained lower amounts of forbs and higher amounts of grasses than the control, and described the composition of

the control more accurately than fecal anslysis. Rumen ingesta showed slightly greater similarity to esophageal samples than to fecal samples.

Fecal analysis was the least accurate method tested. The composition of forbs was lower, and that of grasses higher in fecal samples than in the control esophageal, and rumen samples.

Food habits of large herbivores may be described by using any of the methods tested. Absolute values of species contained in the diets may vary depending upon the method used. Rumen and fecal analysis probably overestimate less digestible portions of the diet while underestimating more digestible portions. Thus, interpretations of diets based on these methods should be made with caution. The esophageal fistula method is probably the most accurate method tested to determine food habits of large herbivores. However, as conditions do not always allow the application of this method, fecal analysis, rumen analysis and ocular-estimate-by-plot techniques are also useful in determining the importance values of species consumed.

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APPENDICES

Precipitation (cm) on the study area.

Year	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
1965	14.5	4.8	1.8	4.6	4.8	4.0	2.2	3.6	3.0	0.5	4.3	2.0	50.3
1966	4.3	6.9	6.6	1.3	3.0	4.0	1.5	0.0	3.6	3.0	6.0	8.3	48.7
1967	7.3	3.3	7.3	9.4	6.4	3.0	1.5	0.0	2.0	5.0	4.0	9.1	58.6
1968	4.8	4.6	5.6	2.3	5.8	3.2	1.3	7.6	6.9	3.0	9.1	8.6	64.2
1969	11.1	11.1	3.8	9.7	5.6	6.6	1.5	0.0	2.8	4.8	2.3	8.6	56.9
1970	9.4	5.8	6.4	4.0	6.4	9.4	2.3	0.0	6.6	7.8	8.9	5.8	72.9
1971	5.6	3.6	10.1	5.0	6.4	5.1	1.3	1.3	4.3	5.6	4.8	13.9	67.0
1972	7.6	4.3	9.4	5.8	2.5	7.6	0.5	1.5	1.8	2.3	6.4	7.6	57.4
1973	3.8	3.3	2.5	2.8	3.0	?	?	1.8	7.1	6.0	10.6	13.7	54.8
1974	7.6	5.3	6.4	7.6	6.1	2.5	4.0	1.3	0.0	0.5	4.6	11.2	57.2
10-yr mean	7.6	5.0	5.8	5.0	5.0	4.6	1.5	1.8	3.8	3.8	6.1	8.9	58.9
1975	15.2	5.0	7.1	9.7	6.9	3.3	8.1	2.5	0.0	7.6	8.1	4.3	77.9

Maximum temperatures ($^{\circ}$C) on the study area.

Year	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
1965	6	15	22	24	30	31	39	27	27	31	24	14
1966	9	15	26	29	36	34	39	39	37	29	17	8
1967	13	17	15	17	32	33	42	41	37	26	17	10
1968	13	19	20	24	28	36	38	29	34	29	13	10
1969	8	12	21	25	31	33	39	38	33	26	20	13
1970	13	16	14	23	29	37	38	40	37	23	12	4
1971	?	6	11	22	28	33	37	39	34	29	13	7
1972	11	14	22	20	33	34	37	39	32	26	13	9
1973	8	14	18	23	31	34	37	38	34	27	12	8
1974	9	11	14	24	23	32	36	29	33	29	14	7
10-yr mean	7	14	18	23	30	34	38	36	34	27	19	9
1975	9	14	16	17	30	27	38	32	35	28	?	8

APPENDIX A (Continued)

Minimum temperatures ($^{\circ}$C) on the study area.

Year	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
1965	-11	-19	-20	- 7	-6	-2	-1	-4	-8	- 3	- 7	-19
1966	-18	-18	-16	- 8	-6	-1	-1	-2	-4	- 8	- 8	-11
1967	-11	-15	-15	- 9	-6	4	3	4	-3	- 6	-14	-24
1968	-18	-13	-11	-12	-6	0	2	1	-2	- 8	- 9	-26
1969	-18	-14	-13	- 8	-6	1	1	-2	-5	-12	- 9	-10
1970	-16	-14	-12	-10	-7	-1	3	-1	-8	-11	-16	-19
1971	-20	-18	-13	- 9	-5	2	-2	0	-7	-18	-23	-22
1972	-24	-10	-12	- 9	-3	-1	0	-1	-4	- 9	- 7	-31
1973	-24	- 8	-10	- 8	-7	-1	1	-1	-4	- 8	-17	- 9
1974	-28	-14	-12	- 5	-4	-1	4	2	-4	- 8	- 7	-20
10-yr mean	-19	-14	-13	- 8	-6	-1	1	0	5	- 9	-12	-19
1975	-18	-14	-18	-10	-4	-2	2	-1	1	-11	?	-18

APPENDIX B

Soil Description for the Wilkens Series
Occurring in the Study Area

Horizon	Depth (inches)	
A11	0-2	Grayish brown (10 YR 5/2 dry), very dark grayish brown (10 YR 3/2 moist) silt loam; moderately thin platy structure that breaks into weak fine granules; friable, slightly sticky and slightly plastic; abundant roots; pH 6.8; clear, smooth lower boundary. 1/2 to 3 inches thick.
A12	2-5	Grayish brown (10 YR 5/2 dry), very dark grayish brown (10 YR 3/2 moist) silt loam; moderate medium platy structure which breaks apart to moderate fine granules; pH 6.4; clear, smooth lower boundary. 2-6 inches thick.
B1	5-11	Grayish brown (10 YR 5/2 dry), drak grayish brown (10 YR 4/2 moist) silty clay loam; moderate medium prismatic structure that breaks to weak medium subangular blocks; firm, sticky and very plastic; many roots; pH 6.3; abrupt lower boundary. 3-10 inches thick.
A2	11-12	Gray (10 YR 5.5/1 dry, 10 YR 5/1.5 moist), silt with weak thin platy structure which breaks apart to weak very fine granules; friable, slightly sticky and slightly plastic; many roots; pH 6.2; abrupt lower boundary. 0-1 ^{1}/2 inches thick.
B2	12-22	Light brownish gray (10 YR 6/2 dry), olive brown (2.57 3/4 moist) clay; moderate fine prismatic structure that breaks into strong very fine blocks; very hard, very firm, very sticky and very plastic; few roots; many thick continuous clay films on vertical and horizontal ped surfaces and root channels; dark grayish brown (2.57 4/2 moist) mottles that are common, fine, and distinct; pH 6.2; clear, smooth lower boundary. 7-12 inches thick.

Appendix B.   (Continued)

Horizon	Depth (inches)	
C	22-27	Olive (5Y 4/2 moist) silty clay loam; massive; dark grayish brown (2.5Y 4/2 moist) mottles that are common, fine, and distinct; none to very few roots; pH 6.6; abrupt lower boundary. 5-8 inches thick.
Dr	27	Very pale brown (10 YR 7/3 dry), olive (5Y 5/4 moist) fine-grained sedimentary rocks.

APPENDIX C

Scientific Names and Common Names of plants Occurring on the Study Area According to the Nomenclature of Hitchcock and Cronquist (1973)

Scientific name	Common name
Graminoids	
Bromus inermis Leys.	Smooth brome
Bromus mollis L.	Soft brome
Bromus tectorum L.	Cheatgrass brome
Carex sp.	Sedge
Danthonia californica Boland.	California danthonia
Festuca idahoensis Elmer	Idaho fescue
Juncus balticus Willd.	Baltic rush
Koeleria cristata Pers.	Prairie junegrass
Luzula campestris (L.) DC.	Field woodrush
Phleum pratense L.	Timothy
Poa compressa L.	Canada bluegrass
Poa pratensis L.	Kentucky bluegrass
Stipa occidentalis Thurb.	Western needlegrass
Forbs	
Achillea millefolium L.	Yarrow
Agoseris glauca (Pursh) Raf.	Pale agoseris
Anenome occidentalis Wats.	Western pasqueflower
Anenome piperi Britt.	Piper anenome
Antennaria microphylla Rydb.	Rose pussytoes
Arnica fulgens Pursh	Orange arnica
Camassia quamash (Pursh) Greene	Common camas
Cirsium vulgare (Savi) Tenore	Bull thistle
Collinsia parviflora Lindl.	Littleflower collinsia
Delphinium occidentale Wats.	Duncecap larkspur
Epilobium paniculatum Nutt.	Autumn willowweed
Equisetum arvense L.	Field horsetail
Erigeron pumilus Nutt.	Shaggy fleabane
Fragaria virginiana Duchesne	Blueleaf strawberry
Galium boreale L.	Northern bedstraw
Geranium viscosissimum F. & M.	Sticky geranium
Geum triflorum Pursh	Prairiesmoke avens
Iris missouriensis Nutt.	Rockymountain iris
Lithophragma parviflora (Pursh) Coult. & Rose	Nineleaf lomatium
Lupinus leucophyllus Dougl.	Velvet lupine
Oenothera subacaulis (Pursh) Garrett	Longleaf eveningprimrose
Penstemon globosus (Piper) Pennell & Keck	Globe penstemon
Potentilla glandulosa Lindl.	Gland cinquefoil

Appendix C. (Continued)

Scientific name	Common name
Potentilla gracilis Dougl.	Beauty cinquefoil
Rumex acetosella L.	Sheep sorrel
Sanguisorba occidentalis Nutt.	American burnet
Saxifraga integrifolia Hook.	Swamp saxifrage
Sidalcea oregana (Nutt.) Gray	Oregon checkermallow
Solidago missouriensis Nutt.	Missouri goldenrod
Taraxacum officinale Weber	Common dandelion
Tragopogon dubius Scop.	Yellow salsify
Vicia amercana Muhl.	American vetch
Viola adunca Sm.	Hook violet

Shrub

Symphoricarpos albus (L.) Blake	Common snowberry

APPENDIX D

Formulae for Hoyer's Mounting Medium and
Hertwig's Clearing Solution
(Ward, 1970)

<u>Hoyer's Mounting Medium</u>

20% gum arabic

35% distilled water

12% glycerin

30% chloral hydrate

3% glucose

<u>Hertwig's Clearing Solution</u>

19 cc HCl added to 150 cc water

60 cc glycerine

270 g chloral hydrate crystals

APPENDIX E

Relations of Frequency to Density (Fracker and Brischle, 1944)

Frequency (%)	Density	Frequency (%)	Density	Frequency (%)	Density	Frequency (%)	Density
1	0.01	26	0.30	51	0.71	76	1.43
2	0.02	27	0.31	52	0.73	77	1.47
3	0.03	28	0.33	53	0.75	78	1.51
4	0.04	29	0.34	54	0.77	79	1.56
5	0.05	30	0.35	55	0.80	80	1.61
6	0.06	31	0.37	56	0.82	81	1.66
7	0.07	32	0.38	57	0.84	82	1.71
8	0.08	33	0.40	58	0.86	83	1.77
9	0.09	34	0.41	59	0.89	84	1.83
10	0.10	35	0.43	60	0.91	85	1.89
11	0.11	36	0.44	61	0.94	86	1.96
12	0.12	37	0.46	62	0.96	87	2.04
13	0.14	38	0.48	63	0.99	88	2.12
14	0.15	38	0.49	64	1.02	89	2.20
15	0.16	40	0.51	65	1.05	90	2.30
16	0.17	41	0.52	66	1.08	91	2.40
17	0.18	42	0.54	67	1.11	92	2.52
18	0.20	43	0.56	68	1.14	93	2.66
19	0.21	44	0.58	69	1.17	94	2.81
20	0.22	45	0.60	70	1.20	95	2.99
21	0.23	46	0.62	71	1.23	96	3.22
22	0.25	47	0.63	72	1.27	97	3.51
23	0.26	48	0.65	73	1.31	98	3.91
24	0.27	49	0.67	74	1.35	99	4.60
25	0.29	50	0.69	75	1.39	100	-

APPENDIX F

Percent Frequency of Occurrence of Species in Each Pasture for Both Grazing Trials

Species	Pastures							
	1	2	3	4	5	6	7	$\bar{x}$
Graminoids								
Smooth brome	0	0	0	0	0	5	5	1
Soft brome	50	95	45	65	70	85	90	71
Sedges[a]	95	65	85	60	80	60	50	71
California danthonia	55	5	20	15	20	15	5	19
Idaho fescue	0	5	0	0	10	0	5	3
Baltic rush	30	55	30	50	35	20	35	36
Prairie junegrass	60	50	90	75	60	45	40	60
Timothy	100	55	40	60	65	90	85	71
Canada bluegrass	5	15	10	0	5	30	30	14
Kentucky bluegrass	100	100	95	95	95	100	95	97
Western needlegrass	35	65	50	55	35	0	5	35
Forbs								
Yarrow	95	85	95	80	90	70	90	86
Pale agoseris	35	90	45	60	65	30	25	50
Western pasqueflower	0	0	0	15	5	0	0	3
Rose pussytoes	55	40	70	40	60	20	35	46
Orange arnica	35	35	60	25	80	0	25	37
Common camas	25	0	0	0	5	5	0	5
Littleflower collinsia	5	5	20	10	30	45	80	28
Duncecap larkspur	5	5	0	0	5	15	0	4
Autumn willowweed	50	60	25	65	0	65	65	47
Field horsetail	20	95	100	40	60	0	20	48
Shaggy fleabane	0	5	0	0	0	0	0	1
Blueleaf strawberry	40	55	30	40	25	25	25	35
Northern bedstraw	30	5	5	30	10	5	15	14
Prairiesmoke avens	20	25	35	40	15	10	45	27
Rockymountain iris	10	5	0	20	10	5	10	9
Nineleaf lomatium	10	15	5	0	0	25	20	11
Velvet lupine	75	70	95	65	90	80	85	80
Globe penstemon	15	5	0	10	15	5	10	9
Gland cinquefoil	10	5	45	15	10	0	0	12
Beauty cinquefoil	90	80	90	85	100	90	90	89
Sheep sorell	30	20	10	10	20	10	25	18
American burnet	0	20	5	30	0	5	40	14
Oregon checkermallow	85	80	45	70	75	55	25	62
Missouri goldenrod	80	90	30	80	80	90	85	76
Common dandelion	55	75	85	75	80	70	90	76
Yellow salsify	20	25	15	30	25	60	35	30
American vetch	70	70	85	45	80	40	30	60
Hook violet	45	55	75	60	40	25	30	47

[a]Sedges not identified to species.

APPENDIX G

Mean Dry Weight Production (pounds per acre) of Each Species in Every Pasture of the Early Summer Grazing Trial

Species	Pastures						
	1	2	3	4	5	6	7
Graminoids							
Smooth brome	0	0	0	0	0	3	5
Soft brome	7	10	10	5	14	23	11
Sedge[a]	170	130	142	114	144	74	44
California danthonia	18	6	5	6	2	6	0
Idaho fescue	0	4	0	0	4	0	2
Baltic rush	8	23	1	15	10	1	10
Prairie junegrass	8	19	25	16	11	9	8
Timothy	94	27	9	20	22	84	76
Canada bluegrass	0	12	2	0	3	8	18
Kentucky bluegrass	92	138	150	100	145	132	100
Western needlegrass	13	19	19	11	7	0	0
Forbs							
Yarrow	54	128	88	34	60	9	33
Pale agoseris	1	46	16	40	15	7	10
Western pasqueflower	0	0	0	2	2	0	0
Rose pussytoes	7	5	6	4	9	2	5
Orange arnica	7	3	17	8	27	0	2
Common camas	0	0	0	0	0	0	0
Littleflower collinsia	t[b]	0	t	5	3	3	9
Duncecap larkspur	t	t	0	0	t	3	0
Autumn willowweed	t	2	16	3	0	3	2
Field horsetail	4	22	17	3	9	0	1
Shaggy fleabane	0	3	0	0	0	0	0
Blueleaf strawberry	34	14	5	22	3	10	6
Northern bedstraw	13	1	1	33	0	1	1
Prairiesmoke avens	13	15	24	34	20	6	0
Rockymountain iris	10	20	0	20	0	0	t
Nineleaf lomatium	0	0	0	0	0	0	0
Velvet lupine	524	278	528	78	130	136	233
Globe penstemon	3	0	0	2	5	0	11
Gland cinquefoil	3	0	5	2	1	0	0
Beauty cinquefoil	320	104	424	426	552	458	490
Sheep sorrel	4	0	t	1	1	t	4
Oregon checkermallow	39	32	32	71	64	35	25
Missouri goldenrod	34	38	8	23	39	41	47
Common dandelion	8	9	8	6	7	10	18
Yellow salsify	1	3	4	11	1	7	8
American vetch	15	26	112	10	14	6	3
Hook violet	11	6	7	4	7	8	2

[a] Sedge not identified to species. [b] Trace. Less than 1 pound per acre.

APPENDIX H

Mean Percent Utilization of Each Species for Early Summer Grazing Trial

Species	Pastures						
	1	2	3	4	5	6	7
Graminoids							
Smooth brome							
Soft brome	0	0	0	0	0	0	0
Sedge[a]	12.3	2.5	1.1	0	2.9	10.0	7.1
California danthonia	10.0		0	0	0	0	0
Idaho fescue					0		
Baltic rush	12.5	13.3	20.4	0	16.6	8.3	2.5
Prairie junegrass	0.7	1.2	1.1	0	0	5.0	6.3
Timothy	19.8	40.8	5.0	3.0	23.3	11.9	19.8
Canada bluegrass	0	2.5				1.3	3.3
Kentucky bluegrass	5.0	11.5	0.5	0.4	0.5	1.5	8.5
Western needlegrass	0	0	0	0	0		10.0
Forbs							
Yarrow	0	0	0	2.4	0	0	0
Pale agoseris	0	9.1	12.5	1.4	5.8	1.5	0
Western pasqueflower				0			
Rose pussytoes	0	8.3	1.4	0	0	0	0
Orange arnica	6.0	3.3	1.1	2.5	0.7		0
Common camas							
Littleflower collinsia		0	0		0	0	
Duncecap larkspur						0	
Autumn willowweed	0	14.1	0	0		2.9	8.3
Field horsetail	27.5	33.3	26.4	3.75	10.0		8.3
Shaggy fleabane							
Blueleaf strawberry	0	1.4	0	0	0	0	0
Northern bedstraw	2.5			0	0		0
Prairiesmoke avens	0	0	0	0	0	0	0
Rockymountain iris				1.6	0	0	0
Nineleaf lomatium							
Velvet lupine	0	0	0	0	0		0
Globe penstemon	0	0				0	
Gland cinquefoil		0	0	0	5.0		
Sheep sorrel	0	0	25.0	0	0		10.0
Oregon checkermallow	0	13.3	0	3.6	4.4	34.2	0
Missouri goldenrod	5.1	8.5		0.6	0	0.6	0.5
Common dandelion	5.8	10.6	1.3	0	0	7.5	2.8
Yellow salsify	0	51.3	0	2.5	15.0	33.3	63.3
American vetch	5.8	8.3	6.9	2.5	2.9	0.7	16.6
Hook violet	1.3	0.8	0	0	0	0	0

[a] Sedge not identified to species.

APPENDIX I

Mean dry weight production (lb per acre) of each species in every pasture of the late summer grazing trial.

Species	Pastures				
	1	2	3	4	5
Graminoids					
Smooth brome	t[b]	0	0	0	0
Soft brome	1	4	2	2	2
Sedge[a]	90	68	110	35	130
California danthonia	3	0	3	6	3
Idaho fescue	0	0	0	0	1
Baltic rush	2	4	0	15	3
Prairie junegrass	7	6	11	2	6
Timothy	174	34	32	23	24
Canada bluegrass	0	4	0	4	0
Kentucky bluegrass	37	46	46	38	34
Western needlegrass	5	1	4	5	4
Forbs					
Yarrow	26	45	66	13	38
Pale agoseris	6	2	2	9	2
Western pasqueflower	0	0	0	0	0
Rose pussytoes	3	t	4	t	3
Orange arnica	3	0	5	t	t
Common camas	0	0	0	0	0
Littleflower collinsia	0	t	0	0	0
Dunecap larkspur	0	0	0	0	0
Autumn willowweed	t	1	t	5	t
Field horsetail	10	13	19	3	3
Shaggy fleabane	0	0	0	0	0
Blueleaf strawberry	17	7	10	23	0
Northern bedstraw	15	0	2	12	0
Prairiesmoke avens	16	2	3	t	6
Rockymountain iris	t	2	7	0	2
Nineleaf lomatium	0	0	0	0	0
Velvet lupine	355	198	387	181	126
Globe penstemon	0	0	1	0	0
Gland cinquefoil	4	0	0	5	1
Sheep sorrel	1	t	2	0	t

Appendix I (Continued)

Species	Pastures				
	1	2	3	4	5
Oregon checkermallow	18	18	42	24	18
Missouri goldenrod	34	31	13	16	13
Common dandelion	1	14	14	0	t
Yellow salsify	2	2	2	14	2
American vetch	5	3	5	3	2
Hook violet	2	1	3	t	t

[a]Sedge not identified to species.

[b]Less than 1 lb per acre.

APPENDIX J

Mean percent utilization of each species for late summer grazing trial.

Species	Pasture				
	1	2	3	4	5
Graminoids					
Smooth brome		15.0			
Soft brome	0	0	0	0	0
Sedge[a]	7.5	11.1	t[b]		
California danthonia			0	0	0
Idaho fescue					
Baltic rush		6.7	19.6	3.5	6.7
Prairie junegrass	4.8	10.9	5.8	0	4.4
Timothy			1.9		
Canada bluegrass		27.5			
Kentucky bluegrass	9.9	14.0	6.2	1.6	9.7
Western needlegrass	0	15.0	3.3	t	0
Forbs					
Yarrow	1.0	0	0	0	0
Pale agoseris	0	5.9			
Western pasqueflower					
Rose pussytoes	2.1	1.7		2.0	0
Orange arnica		0	1.8	2.5	3.3
Common camas					
Littleflower collinsia		0		0	0
Dunecap larkspur					
Autumn willowweed	0		0	t	2.0
Field horsetail	27.5	25.0	9.7	23.8	2.1
Shaggy fleabane					
Blueleaf strawberry	3.8	8.6	1.3	0	0
Northern bedstraw				2.5	
Prairiesmoke avens	0	0	t	0	1.7
Rockymountain iris	2.5			3.3	2.0
Nineleaf lomatium					
Velvet lupine	1.0	16.0	1.7	0	t
Globe penstemon	0		0	11.7	
Gland cinquefoil		0			
Sheep sorrel	0	10.0			12.5

Appendix J. (Continued)

Species	Pasture				
	1	2	3	4	5
Oregon checkermallow	14.3	6.7	0	3.4	6.4
Missouri goldenrod		1.1			
Common dandelion	t			0	0
Yellow salsify	0		55.0	9.3	42.5
American vetch					
Hook violet		4.3	1.3	0	0

[a]Sedge not identified to species.

[b]Less than 1%.