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GARY EUGENE SMITH for the M. S. in ANIMAL NUTRITION
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The objectives of this study were to determine the nutritional values of big sagebrush (Artemesia tridentata), to detect possible deleterious effects on animal utilization from the essential oils in sagebrush, and to determine the changes in the chemical composition of big sagebrush, as affected by seasonal differences and fertilizer application. While application of the data to deer was eventually contemplated, the investigations were largely carried out with sheep, or in the laboratory.

Two in vivo digestion trials were conducted using alfalfa and a mixed-grass hay fed singly and in combination with various percentages of sagebrush. A third trial included pelleted alfalfa hay fed singly and in combination with various percentages of sagebrush which was forced into the rumen via fistulas.

Consumption of sagebrush by lambs varied from 75 to 306 grams per day compared to 500 to 900 grams per day for the other forages. Body weight changes were not adversely affected by the amounts of sagebrush fed in these trials.

Mean digestibility coefficients for dry matter (54.5%), energy (53.1%), ether extract (72.3%), and nitrogen-free extract (59.2%) of sagebrush were consistent in all digestion trials. Whereas, mean digestibility coefficients for protein (54.0%) and crude fiber (29.1%) were highly variable.

An artificial rumen was used to study the effects of essential oils on microbial digestion. Basically, this in vitro procedure involved incubating substrates for 24 hours with 15 milliliters of rumen fluid and 30 milliliters of mineral solution, to which various levels of essential oils were added, in glass centrifuge bottles immersed in a water bath held at a constant temperature of 39° C.

A selective effect from these essential oils is suggested since in vitro dry matter digestibility of sagebrush was not adversely affected by even the 100 µl levels of oil supplementation, whereas, all other substrates were significantly affected by the 50 µl level. The yield of essential oils was determined for 18 samples of sagebrush, and ranged from 11.2 to 33.0 µl/gram.

Several factors were employed to evaluate the versatility of the artificial rumen technique used in this study. The mean,

standard deviation, and coefficient of variation were calculated for each of the 384 treatment combinations of substrate, rumen fluid, position of flask, and source of inoculum. Results from the analysis of variance indicate that forages of varying nutritional value may be differentiated by in vitro dry matter digestibility. Furthermore, a procedure which is repeatable has been used with inoculum from both sheep and cattle. Increasing the level of rumen liquor from 5 to 15 ml resulted in greater dry matter digestibility, but to a greater degree with inoculum from cattle than from sheep.

Significant correlation coefficients from nine substrates were obtained between in vitro dry matter digestibility and in vivo digestibilities of energy, dry matter, organic matter, and protein; simple regression equations are given for each of these entities as obtained with 5 and 15 ml of rumen fluid from both sheep and cattle.

The in vitro and predicted in vivo values, obtained from microbial fermentation of monthly sagebrush twigs, suggest that they should be quite highly digestible by ruminants.

Three fertilized and three unfertilized plots of sagebrush were established near Silver Lake, Oregon. Monthly analyses of clippings from these plots showed that nitrogen and phosphate fertilization resulted in an increase in crude protein, in vitro dry matter digestibility and growth; whereas, the percent nitrogen-free

extract was decreased. Apparently, fertilization did not affect the percentages of cellulose, crude fiber, ash, calcium, and phosphorus.

The intensity of winter use of tagged sagebrush twigs by deer was greater on fertilized than unfertilized plots.

NUTRITIONAL EFFECTS OF BIG SAGEBRUSH (ARTEMESIA TRIDENTATA) NUTT. ON DEER

by

GARY EUGENE SMITH

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Professor of Animal Science

In Charge of Major

Redacted for privacy

Head of Department of Animal Science

Redacted for privacy

Dean of Graduate School

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INTRODUCTION

The Bureau of Land Management plans to spray over 100, 000 acres of sagebrush (Artemesia tridentata Nutt.) rangeland in the state of Oregon in 1963 (105, p. 4). Their main objective is to enhance livestock production by increasing the quantity and quality of grass species through the elimination of sagebrush which competes with grasses for soil nutrients. However, appreciable quantities of sagebrush will remain available for big game on certain winter ranges when other more palatable plants have been used up. It has been observed that during severe winters, death losses are heavy for deer which may be due to starvation or perhaps to some unidentified toxic factor present in sagebrush.

Methods for determining the nutritive value of sagebrush and other range plants have been developed by several range investigators (37, p. 579-590; 102, p. 289; 18, p. 78). Digestibility coefficients for sagebrush have been determined in digestion stalls with deer by Smith at Utah (102, p. 289), Bissel at California (18, p. 78), and Dietz at Colorado (51, p. 1-89). In all of these trials consumption of sagebrush was below that necessary to maintain body weight.

Cook et al. (37, p. 579-590) have reported relatively low metabolizable energy values for sagebrush due to the loss of essential oils in the urine. These essential oils are a mixture of steam-volatile oils whose specific chemical activity has not been ascertained. Bissell (18, p. 57-78) has suggested that essential oils present in sagebrush may be deleterious to rumen microorganisms.

Since digestibility data are difficult to obtain under practical range environments, it is plausible that a simple, accurate, and repeatable laboratory procedure would be advantageous. Before a procedure is used, however, it should be standardized with known in vivo data so that its application to unknown samples will be well founded. Such a procedure employs the artificial rumen which is a laboratory apparatus that utilizes rumen microorganisms for the fermentation of substrates. It may be used to evaluate these substrates or to study specific factors affecting microbial activity, such as the effects of sagebrush essential oils on microbial multiplication.

The first section of this thesis includes the development and application of an artificial rumen procedure, and the second section the nutritional evaluation of sagebrush. The objectives of this latter study are as follows: (1) to determine the nutritive value of big sagebrush for ruminants, (2) to determine effects of ingestion of

big sagebrush upon the nutritive value of associated forages, and (3) to determine changes in the chemical composition of big sagebrush throughout the year, as affected by seasonal differences and fertilizer application.

Part I. ARTIFICIAL RUMEN TECHNIQUES AND APPLICATIONS

REVIEW OF LITERATURE

Artificial Rumen Techniques

In recent years there has been increasing interest in the biochemical and microbiological aspects of the rumen. Because of this interest, various artificial rumen procedures have been used to study forage digestibility. In vitro fermentations have also been utilized to investigate various factors influencing digestion in the intact rumen. Basically an artificial rumen, in vitro technique, includes incubation of rumen microorganisms on a substrate for a given length of time, which results in fermentation of that substrate.

One of the first in vitro procedures, in which whole rumen contents were incubated in an impermeable system, was reported by Pearson and Smith in 1943 (88, p. 142-148). Since this modest start, various apparatus have been used to study microbial action. Generally, such apparatus fall into one of three broad classifications: (1) all-glass, impermeable; (2) semipermeable membrane; and (3) continuous flow.

Probably the first all-glass system was developed by Marston in 1948 (81, p. 564-574). Under the environmental conditions,

which were similar to those in the normal rumen, cellulose fermentation *in vitro* by rumen microorganisms yielded the following accumulated end products: large quantities of acetic and proponic acid, methane, and carbon dioxide; smaller quantities of acetaldehyde and formic, butyric, pyruvic, and lactic acids.

Burroughs *et al.* (25, p. 672-682; 26, p. 693-705; 27, p. 9-24) have used an all-glass artificial rumen to study cellulose digestion. Rumen inoculum was diluted each day so that nutrients originally present in the rumen fluid were gradually exhausted.

Bentley and associates in 1954 (17, p. 581-593) developed a simplified all-glass apparatus, with an elaborate mineral medium, to study the requirements of cellulolytic microorganisms. Ohio workers have used similar procedures to study the effect of particle size on cellulose digestibility, effect of amino acids on increasing the rate of cellulose digestion, and the mechanism involved in starch fermentation (45, p. 1098-1109; 44, p. 15-27; 87, p. 414-422).

Other workers (76, p. 867-872; 74, p. 199-208; 92, p. 275-287) have used procedures modified after that used in Ohio (17, p. 581-593) to compare *in vivo* and *in vitro* cellulose digestion. Baker *et al.* (5, p. 655-662) examined forages of varying cellulose

digestibility in vitro with an x-ray diffractometer. The availability of nitrogen to rumen microorganisms was investigated by Hershberger et al. (66, p. 770-779; 67, p. 663-670).

In a series of reports, Donefer, Crampton, and Lloyd (53, p. 1538; 55, p. 815-818; 54, p. 545-552) describe a procedure modified after reports by Bentley et al.(17, p. 581-593) and Quicke et al. (92, p. 275-287). Crampton's group has developed a prediction equation for the Nutritive Value Index (NVI) of forages from their in vitro cellulose digestibilities.

In vitro silage fermentation rates were studied in an all-glass method designed by Barnett in 1957 (6, p. 467-474). Carbon dioxide was bubbled through 100 ml centrifuge tubes connected in series so that anerobic conditions could be maintained for studying the rates of fermentation (6, p. 467-474; 8, p. 171-179; 9, p. 180-183). Levels of fertilization and stages of maturity have been studied in similar types of artificial rumens (68, p. LX-1-LX-6; 110, p. LXV-1-LXV-6).

The artificial rumen developed at the Oregon Station was patterned after an earlier report by Barnett (6, p. 467-474). Church and Petersen in 1960 (32, p. 81-92) studied several variables that affected the in vitro fermentations. Subsequently, this in vitro procedure has been used to study volatile fatty acid production,

total gas production, cellulose digestion, and dry matter digestion of various substrates (31, p. 28;12, p. XLIII-1-XLIII-6; 20, p. 972-979; 21, p. 980-985; 19, p. 1-87; 85, p. 1-60; 98, p. 1-65). This procedure is basically the same as that used in this thesis.

Louw and associates in 1949 (79, p. 478-480) found that a semipermeable membrane apparatus permitted better cellulose digestion than a previously reported impermeable system (81, p. 564-574). This increased digestibility of cellulose was attributed to the removal of inhibitory end products by the semipermeable system. Baumgardt and associates, 1962, (14, p. 62-68) compared the all-glass system and the semipermeable membrane apparatus, but no appreciable differences were found in cellulose digestibility or volatile fatty acid patterns. For a detailed review of the semipermeable systems currently in use, see Walker, 1959 (109, p. 192-197).

Davey and associates (43, p. 155-163) in 1960 described a continuous flow artificial rumen which was shown to simulate closely the conditions in the bovine rumen. Criteria for its validity included quantitative and qualitative bacteriological data, volatile fatty acid production, pH levels and digestion rates. An important feature of this complex continuous flow system is the high degree of control which can be exercised during prolonged

fermentations. The Ohio workers (56, p. 1445-1451) developed a continuous flow apparatus patterned after the semipermeable system suggested by Warner (111, p. 733-748). No major differences were noted when the all-glass, semipermeable membrane, and continuous flow systems were compared in fermentation studies by Cheng and associates (56, p. 1445). Cellulose digestion, total volatile fatty acid production, and ammonia production were the criteria used to compare the three systems. An important advantage was the simplicity of the all-glass system. However, for prolonged periods, greater than 70 hours, the continuous flow system may be advantageous since changes in microflora are less evident if the mineral medium is changed.

Variables in Artificial Rumen Techniques

The artificial rumen used in this thesis is not an attempt to simulate exactly the conditions in the rumen; conversely it is intended to yield "empirical results" which can be used to differentiate forages of varying nutritive value. Therefore, it is essential to standardize conditions of *in vitro* fermentations for the purpose of evaluating feedstuffs and making comparison between them. The variables which will be discussed in this section are the following: (1) criteria for evaluating digestibility, (2) source and preparation

of inoculum, (3) concentration of rumen fluid, and (4) length of fermentation.

Criteria for Evaluating Digestibility

The method for determining the nutritive value of forages may vary in complexity from the energy balance appraisal to simple digestibility trials. Walker (109, p. 192) states that dry matter digestion determined by the latter method is the most useful single measure of the nutritive value of a forage. Even this simple determination of dry matter digestion takes about 20 days with at least three animals. Since rumen fermentation accounts for 50-70% of the total dry matter disappearing in ruminant animals (89, p. 419; 29, p. 525-536) a method for predicting in vivo dry matter digestibility from some in vitro system should be directly applicable to forage evaluation.

Dry Matter digestibility. Baumgardt and Hill in 1956 (12, p. 943) illustrated the importance of controlling various factors affecting dry matter digestibility so that error and bias would not influence results. Baumgardt and associates in 1958 (10, p. 1205) predicted in vivo dry matter digestibility from the in vitro dry matter digestibility of eleven forages (three alfalfas and eight grasses). Examples of unsolved problems which are pertinent to the appraisal of artificial rumen assays were exemplified through dry matter

digestibility in vitro of various forages by Asplund et al. (3, p. 171).

Seasonal changes in the dry matter digestion of timothy stems and leaves were studied by Clark and Mott, 1960, (34, p. 123-129). Methods of drying herbage for the in vitro digestion experiments were also studied with the conclusion that there was a significant difference in favor of freeze-drying over oven-drying (170° F.).

The effects of several variables on in vitro cellulose and dry matter digestion were reported by Church and Petersen, 1960,(32, p. 81-92). Furthermore, in vivo digestibility of forages could be appraised just as accurately by in vitro dry matter digestion as in vitro cellulose digestion.

More recently McNeil, 1962, (85, p. 49-50) studied the effects of time and amounts of rumen fluid on the dry matter digestion in vitro of alfalfa, crested wheatgrass, and brome grass. He also compared three artificial rumen procedures, each of which exhibited some specific characteristic.

Other criteria. Percent cellulose digestibility in vitro has been used as a criterion for evaluating forages by several investigators (70, p. 328; 40, p. 538-544; 54, p. 549; 30, p. 1225-1230). Manometric methods have also been used to determine rumen fermentation ratio (71, p. 199; 24, p. 1-52; 59, p. XLV-6). McNeil (85, p. 1-60) has completed an excellent review of both cellulose

digestibility and manometric determinations.

Source and Preparation of Inoculum

The efficiency with which cattle and sheep digest various feeds has been considered to be essentially the same by many investigators. In a cooperative study undertaken by the Experiment Stations of Maryland, Massachusetts, New Hampshire, New Jersey and Pennsylvania, the apparent digestibility of the dry matter, protein and energy of 28 lots of forages were determined with cattle and with sheep. A statistical study of the data showed no significant difference at the five percent probability level in the digestion capability of sheep and cattle (106, p. 5). An earlier report by Forbes and Garrigus (58, p. 361) suggested that steers and wethers (grazing on various grasses and legumes) exhibited similar digestive capacities for organic matter and protein, and these findings substantiated the results of Jordan and Staples (73, p. 236-243), but there is also information to the contrary. Of particular significance is the report by Cipolloni et al. (33, p. 337-343) who ran statistical analysis of published data.

Cellulose digestion coefficients that were obtained in vitro with sheep and cattle rumen fluid as inoculum were similar. It appeared that sheep and cattle could serve interchangeably as sources of inoculum if the rations fed were similar (76, p. 870-871).

Apparently most methods currently used for rumen fluid preparation are modifications of the following procedures: (1) undiluted rumen liquor incubated with substrates in an all-glass system as reported by Pearson and Smith in 1943 (86, p. 153-164); (2) whole rumen fluid diluted with mineral solution, incubated with substrate in an impermeable system, Burroughs et al. in 1950 (27, p. 9-24); (3) whole rumen liquor incubated with a substrate in a semipermeable container, dialyzing against a mineral solution, Louw et al., 1949 (79, p. 478-481) and Huhtanen, Saunders and Gall in 1954 (70, p. 328-335); and (4) various fractions of rumen liquor, such as centrifuged cells and washed cell suspensions, used in an impermeable system by Bentley et al. in 1954 (17, p. 581-593) and Cheng, Hall, and Burroughs in 1955 (30, p. 1225-1230). Little difference was noted by Quicke et al., 1959, (92, p. 286) when cellulose digestion was measured using strained rumen juice, a phosphate buffer extract of pressed rumen contents or resuspended ruminal microorganisms as inoculum.

Strained rumen liquor, diluted with a mineral solution, was used successfully to digest silage in vitro by Barnett (6, p. 467-474). Church and Petersen (32, p. 81-92), Bowden and Church (22, p. XLIII-1-XLIII-6; 20, p. 972-979; 21, p. 980-985) and McNeil (85, p. 49-50) have extended the use of strained rumen

fluid in determining dry matter digestion with various grass and legumes substrates.

Concentration of Rumen Fluid

The volume of rumen juice, within wide limits, did not affect the percent dry matter digestibility in the artificial rumen procedure designed by Walker (109, p. 196). Earlier Huhtanen and Elliot (69, p. 1183) reported that one-fifth dilution of the original fluid had no effect while further dilutions resulted in progressive diminuation of activity.

In 1960, Church and Petersen (32, p. 86) observed a linear increase in percent dry matter digestion in vitro as the volume of whole rumen fluid was increased from 20 to 120 ml in a system with a total volume of about 750 ml. This increase was affected by both the mineral and substrate concentrations.

Recently, McNeil (85, p. 40), using a 24-hour digestion period, found a significant increase in dry matter digestion with three forages when five ml of rumen juice was compared to 15, 25, and 35 ml. The latter three levels showed no significant differences. This information tends to confirm that reported previously by Cheng, Hall, and Burroughs (30, p. 1229) with washed cell suspensions and purified cellulose as the substrate.

Length of Fermentation Period

Length of fermentation *in vitro* has been one of the most common variables studied. Donefer, Crampton, and Lloyd, 1961, (54, p. 545-552) determined cellulose digestion *in vitro* after 3, 6, 12, 24, and 48-hour fermentation periods in an effort to predict relative intake and energy digestibility. Lag periods in early stages of cellulose digestion appear to be related to forage species; measurable cellulose breakdown in grasses lagged approximately three hours behind that of legumes. These lag periods are in agreement with reports by Quicke *et al.* (92, p. 275-287) and Baumgardt, Taylor, and Cason, (14, p. 66), who report maximum cellulose digestion of alfalfa was reached at 42 hours whereas it was not reached at 48 hours with grasses. Lag periods were reflected in the 12-hour cellulose digestion determination, which was related to relative intake (54, p. 549). However, Reid *et al.* (95, p. 1312) found no consistent relationship between relative intake and cellulose digestion at any of the time periods studied (4, 8, 12, 20, 32, and 48-hours).

Dry matter digestion was determined after 6, 12, 18, 24, 30, 36, 42, and 48-hour fermentation periods in a comprehensive study by McNeil, 1962, (85, p. 49). His data suggest that of the eight time periods studied the 12-hour period may provide the best

means of assessing the digestibility of forages. Differentiation between the digestion of alfalfa and grass samples was more evident at the 24-hour than the 48-hour digestion period.

In Vivo and In Vitro Correlations

A rapid procedure for predicting the nutritive value of forages would be a valuable tool not only to the animal scientist, but also the range manager. Therefore, data obtained from a given artificial rumen procedure should be closely related to various digestibility functions in the animal.

Significant correlations between in vivo and in vitro dry matter digestibilities have been reported by Asplund et al. (3, p. 176), Reid et al. (94, p. 1538), and Clark and Mott (34, p. 123). In vitro procedures reported by Barnett (6, p. 467-474) and Pigden and Bell (91, p. 1239-1240) furnish estimates of in vivo digestibility for crude fiber and total digestible nutrients.

Baumgardt and associates (14, p. 62-68) have recently compared the predictability of several in vitro procedures. By selecting the best method, these New Jersey investigators obtained significant correlations between percent in vitro cellulose digestion and digestibilities in vivo of total digestible nutrients, digestible dry matter and digestible energy (14, p. 62).

Bowden and Church (21, p. 982) showed a high correlation between in vivo digestibilities of dry matter, energy and cellulose and in vitro digestibilities of either dry matter or cellulose. It was also concluded that the procedure for determining in vitro dry matter digestibility was apparently as accurate as in vitro cellulose digestibility for estimating the in vivo digestibility of forages. The series of papers by Bowden and Church (20, p. 972-979; 21, p. 980-985) provide an excellent review and application of correlations between in vitro and in vivo correlations.

Part I. ARTIFICIAL RUMEN TECHNIQUES AND APPLICATIONS

EXPERIMENTAL PROCEDURE

The literature review has emphasized certain variables which should be controlled before empirical data from the artificial rumen will be valid for predicting nutritive values. Satisfactory prediction equations have been reported in the literature; so it seems plausible that similar equations can be developed in this work.

The objectives in this section are as follows: (1) to develop a standardized artificial rumen procedure; (2) to develop equations for predicting digestion coefficients for dry matter, organic matter, energy, protein, and cellulose; and (3) to test the validity of the equations for energy digestibility with additional forages of known *in vivo* digestible energy.

Substrates

All substrates used *in vitro* fermentations had previously been used for determination of digestion coefficients in digestion stalls with wether lambs. Details of the trials with the four meadow grass hays have been presented by Raleigh and Wallace (93, p. 9-12). Digestion coefficients for the alfalfa hay, alfalfa (green chop), and mixed grass hay were obtained with four wether lambs in conventional stalls at the Oregon Station in the summer of 1962. Schubert

et al. (99, p. LV-1-LV-6) reported details of digestion data for the fescue samples. Digestibility data for the ryegrass screenings were reported by Snyder (104, p. 23-24).

Table 1. In vivo digestibilities of the substrates used in this study, in Percent.

	Percent (%) Digestibility				
SUBSTRATE	Dry Matter	Crude Protein	Cellulose	Organic Matter	Energy
Alfalfa Hay	59.5	72.3	55.0	60.8	59.8
Alfalfa (green chop)	62.7	77.7	70.1	67.1	65.1
Meadow Grass Hay 1	61.8	63.0	68.0	63.3	60.3
Meadow Grass Hay 2	56.6	60.2	59.8	58.2	55.8
Meadow Grass Hay 3	51.7	48.4	55.1	53.5	50.5
Meadow Grass Hay 4	49.2	35.2	54.0	51.1	47.8
Fescue	76.4	69.3		79.4	75.5
Mixed Grass Hay	62.6	45.4	69.0	65.1	60.5
Sagebrush	53.9	32.2	44.0	53.9	50.3

Table 2. Chemical composition of the substrates used in this study, in Percent.

	Percent (%)						
SUBSTRATE	Dry Matter	Crude Protein	Cellulose	Organic Matter	Crude Fiber	Ether Extract	Ash
Alfalfa Hay	98.1	16.4	28.0	87.6	29.0	1.6	10.5
Alfalfa (green chop)	95.6	23.5	22.0	84.3	19.1	2.9	11.3
Meadow Grass Hay 1	94.7	10.5	33.5	87.4	28.6	2.5	7.3
Meadow Grass Hay 2	94.7	8.7	35.6	87.0	30.3	2.4	7.7
Meadow Grass Hay 3	95.0	6.0	35.1	87.0	31.6	2.4	8.0
Meadow Grass Hay 4	95.1	5.0	37.4	87.2	32.1	2.5	7.9
Fescue	94.7	12.2		90.1	24.2	2.2	7.5
Mixed Grass Hay	95.3	6.5	33.5	89.3	32.6	2.5	6.0
Sagebrush	95.9	8.4	26.8	91.3	22.3	4.6	4.6

Digestion coefficients for the sagebrush were obtained by difference with alfalfa hay (green chop) using four wether lambs in conventional digestion stalls. The substrates were dried, chopped through 40 mesh screen in a Wiley mill, and placed in tightly sealed bottles until digested in the artificial rumen. The in vivo digestion coefficients and the chemical composition of the substrates are listed in Tables 1 and 2.

Fermentation Apparatus

The in vitro apparatus, developed for use in this study, was patterned after a modification of the artificial rumen system reported by Barnett and Reid (7, p. 315-316) which was first used in this laboratory by Church and Petersen (32, p. 81). One gram of substrate was incubated in 72, 250 ml centrifuge bottles which were held in two water baths maintained constant at 39° C. These bottles were connected in a series of four with looping rubber tubing attached to polyethylene tubing that was inserted through two holed rubber stoppers. Figure 1 shows the arrangement of fermentation flasks which allows continuous flow of CO_2 throughout the incubation period.

Artificial Saliva Solution

Thirty ml of mineral solution or buffer patterned after that of

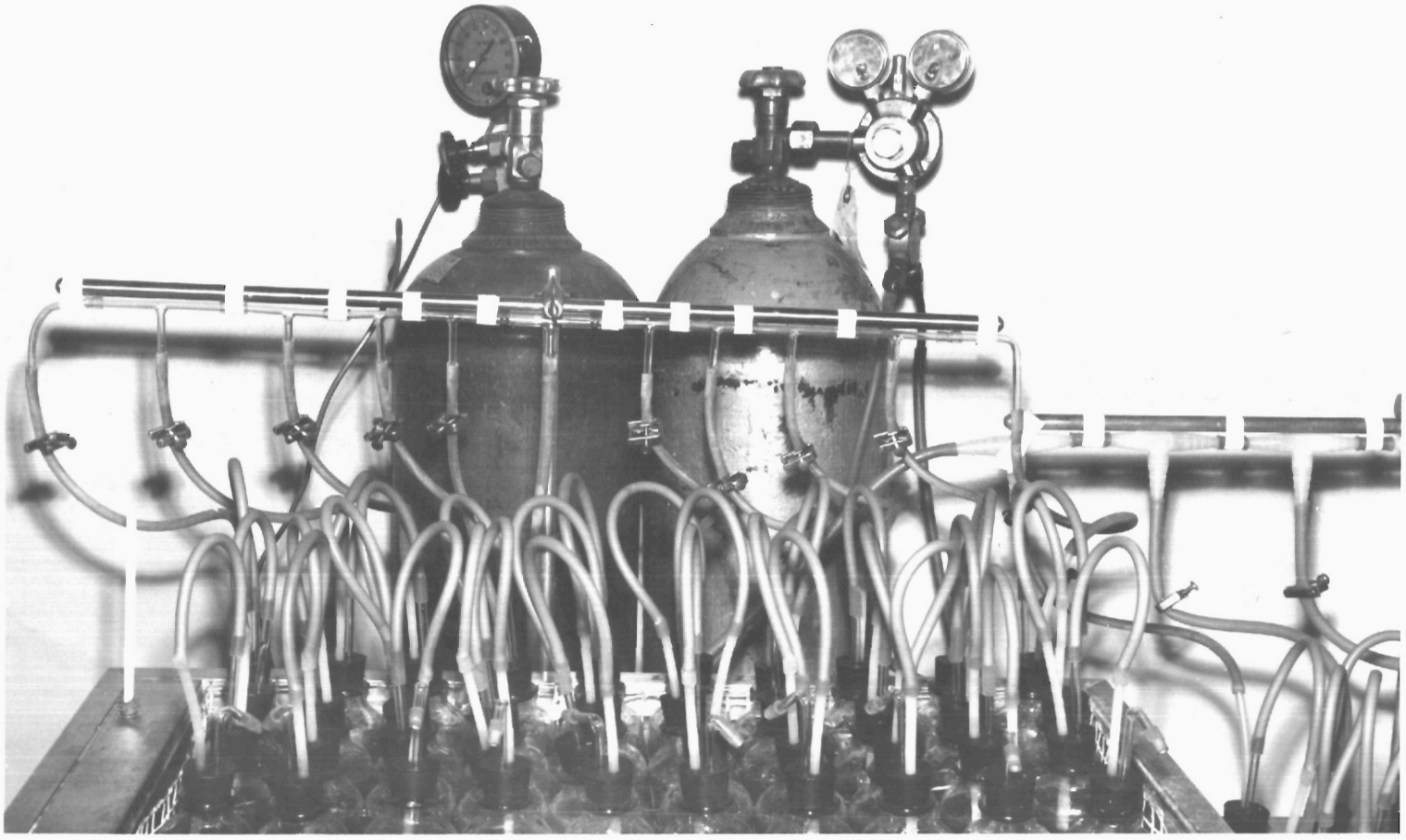

Figure 1. Artificial rumen for in vitro digestion. Note fermentation bottles, constant temperature water bath, connections between fermentation bottles, and connections to the carbon dioxide tank.

McDougall (85, p. 106) had been used satisfactorily by Bowden and Church (20, p. 973) in this laboratory. This mineral solution is basically the same as that used in this study except for the additional urea at the level suggested by McNeil (85, p. 21). Thirty ml of the mineral solution were made up to a total volume of 50 ml (with appropriate amounts of rumen fluid and water) whereas, a total volume of 100 ml was used by the previous investigators (20, p. 973; 85, p. 21). The artificial saliva was composed of the following:

Salt	(g/liter)
$NaHCO_3$	4.17
Na_2CO_3	8.30
$Na_2HPO_4 \cdot 12H_2O$	1.99
KCl	.48
$CaSO_4 \cdot 2H_2O$	.04
$MgSO_4$	.002
Urea	.64
	15.622

Three liters of fresh mineral solution were prepared the evening before each run and placed in a drying oven at 39° C. Just before the buffer was used carbon dioxide was bubbled through it reducing the pH from 8.6 to 7.1.

Rumen Fluid Inoculum

Two Hampshire wether lambs and an adult Hereford steer,

all with rumen fistulas, provided the inoculum for this work. Pelleted alfalfa hay was fed to both species while water and salt were supplied ad libitum. Three liters of rumen fluid were collected around 7:30 a.m. prior to the morning feed, which was approximately 15 hours after the last feed. Rumen contents from the steer were squeezed by hand into previously-heated quart thermos bottles. Rumen liquor from each fistulated lamb was collected by gravity flow into a 600 ml polyethylene beaker and quickly poured into the thermos bottles. The fluid was transported immediately to the laboratory where it was filtered through four layers of cheesecloth into tall beakers. After standing for about 15 minutes at 39° C, the bottom layer was removed by suction. This inoculum was immediately mixed with warm distilled water and buffer solution (pH 7.1) in the following proportions:

5 ml rumen fluid/flask		15 ml rumen fluid/flask
200 ml	Rumen Fluid	600 ml
800 ml	Distilled Water	400 ml
1200 ml	Mineral Mix	1200 ml
2200 ml		2200 ml

The inoculum was poured into an automatic pipette (SGAC) which delivered 50 ml of mixed inoculum into each of the flasks (Figure 2). The pipette had an Eastern model 1 stirrer attached to keep the solution thoroughly mixed, and carbon dioxide was

Figure 2. Inoculation of substrates. Notice the automatic pipette containing the inoculum, fermentation bottle, electric stirrer, carbon dioxide tube, pH meter, and artificial rumen.

then bubbled through the flasks to stir the contents and maintain the desired pH (Figure 3).

Dry Matter Determination

After 24 hours of fermentation the contents of each flask were filtered through previously weighted sintered glass crucibles (50 ml pyrex, 40 μ pore size) under suction (Figure 4). Hot water was used to remove the residue that clings to the glass inlet tubes and to rinse out the flasks. A rubber policeman on a glass stirring rod was used to loosen particles which stuck to the side of the flasks. The crucibles were then dried for 18 hours at 110° C, cooled for two hours, and weighed. Residue remaining in the crucibles was considered to be the undigested dry matter of each substrate. Percent of dry matter (D M) digestibility was calculated as follows:

$$\% \text{ D M Digestibility} = \frac{\text{Weight D M digested}}{(\text{Weight of Sample D M}) + (\text{Weight D M in Inoculum})}$$

It is important that the dry matter in the inoculum be included since the dry matter will vary with each collection of rumen fluid (5 - 30 mg); there is more dry matter in 15 ml of rumen liquor than in 5 ml of rumen fluid. The crucibles were cleaned in about two days with a sulfuric acid-potassium dicromate cleaning solution. It is important to weigh the crucibles after each run since

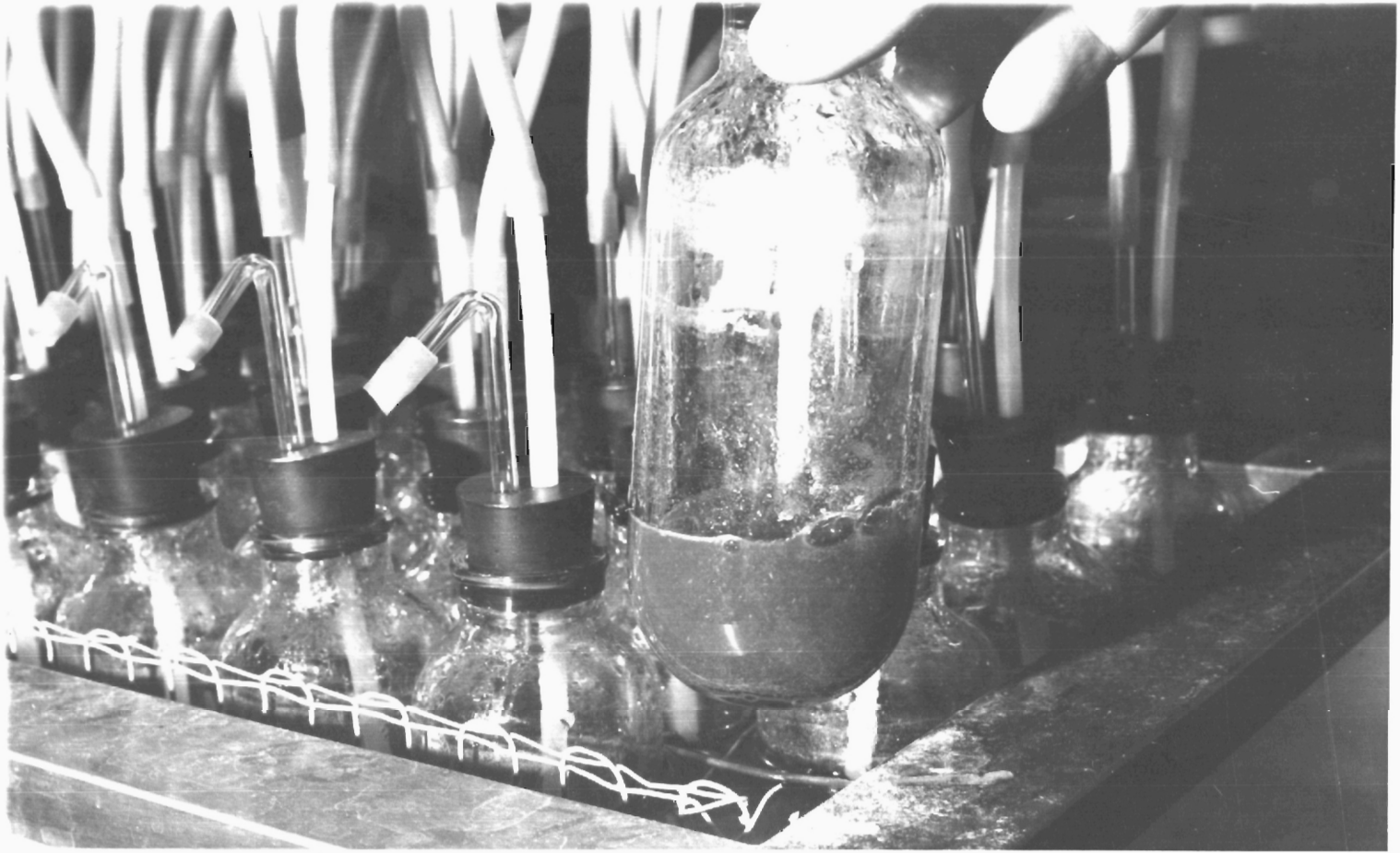

Figure 3. Individual fermentation bottle which shows carbon dioxide bubbling through the contents.

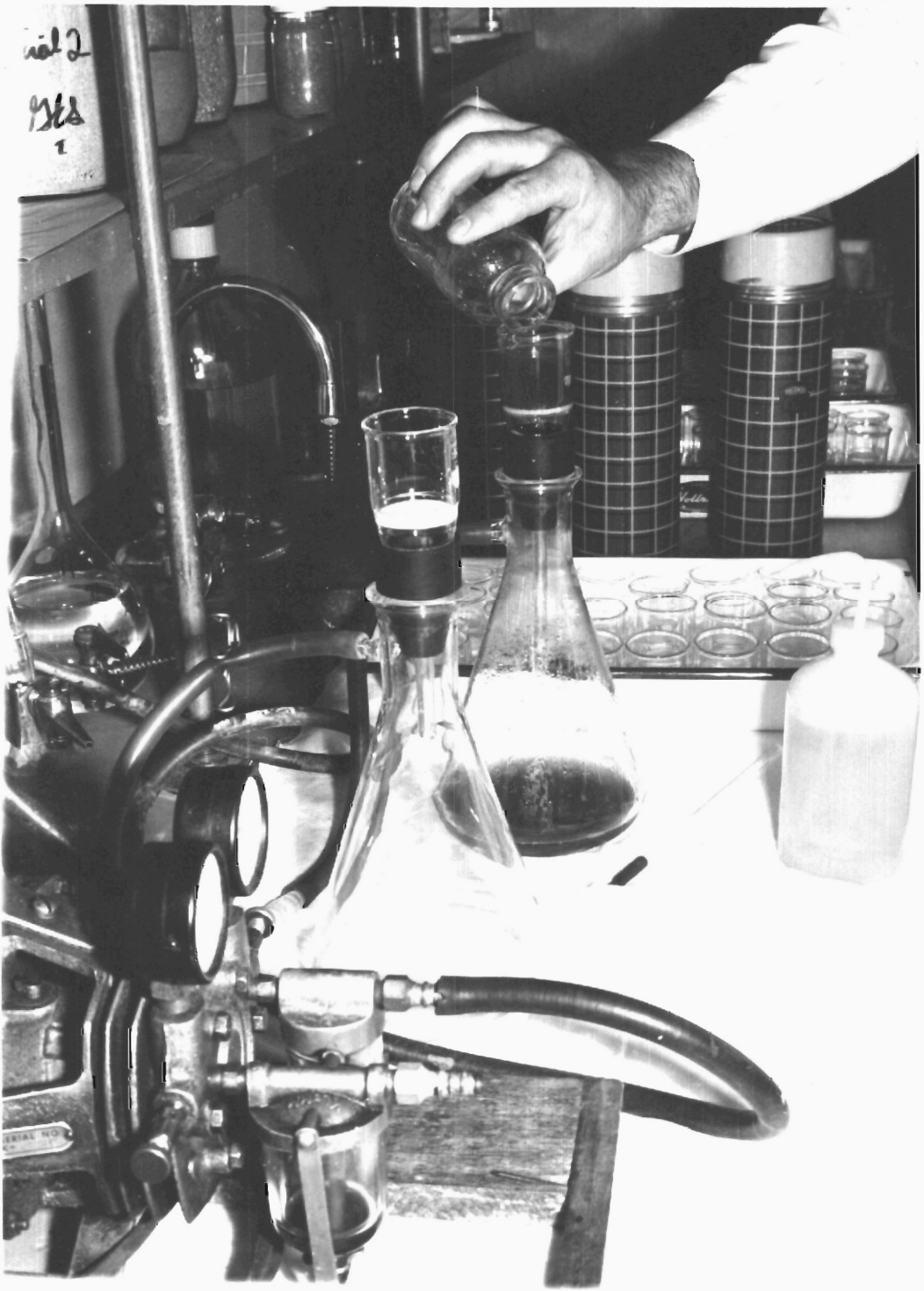

Figure 4. Collection of undigested dry matter. Notice especially the sintered glass crucibles, suction flasks, suction pump, and wash bottle.

some dry matter might be left in the sintered glass mat.

Experimental Designs

A split-split-plot design as defined by Cochran and Cox (35, p. 293-316) was used to evaluate the effects of several variables. Then prediction equations were developed, and tested for their reliability.

Standardization Procedure

A split-split-plot design was used to compare the effects from the following factors:

Factors	Comparisons
Whole Plot:	
3 Replications	Day to day variation
2 Inoculums	Inoculum from cattle and sheep
Sub Plot:	
2 Levels	5 vs. 15 ml of rumen liquor
8 Substrates	Alfalfa hay, alfalfa (green chop), sagebrush, mixed grass hay, and meadow grass hays 1, 2, 3, 4
Sub-Sub Plot	
4 Locations	4 positions of bottles in the water bath

In this design the whole plot factors are less precisely estimated than those for the sub plot or the sub-sub plot (35, p. 293-316). However, the interactions are precisely estimated.

Development of Prediction Equations

Mean in vitro dry matter data from the standardization

procedure were correlated with the various factors presented in Tables 1 and 2. Methods for statistical analysis as described by Li (77, p. 244-268) and Snedecor (103, p. 122-193) were used for the regression equations and correlations.

Reliability of Regression Equations

Twelve random substrates with known digestible energy were digested in the artificial rumen to test the reliability of the regression equations. Calculated *in vivo* digestible energy values were compared to the actual digestible energy values using the Student's t-test as described by Li (77, p. 132).

Part I. ARTIFICIAL RUMEN TECHNIQUES AND APPLICATIONS

RESULTS AND DISCUSSION

Standardization Procedure

The purpose of standardizing the *in vitro* procedure was to develop a reliable procedure which exhibited a minimum amount of variation both between and within runs. In so doing, a split-split-plot design was used to evaluate the effects from several factors.

Variability of Procedure

The means, standard deviations, and coefficients of variation are shown in Tables 3 and 4 for rumen fluid levels, replications, species, and substrate variables imposed on the artificial rumen system. The means for each replication or run are an average of four observations whereas the pooled means are averaged from 12 observations. The variation associated with run means is a reflection of the within trial differences. This variation is about the magnitude that would be expected, however there was less variation in digestion with inoculum from the lambs than from the steer. In addition to the variation from the inoculum, undoubtedly there is a certain amount of error due to experimental procedure. Standard deviations for the means range from .13 to 3.83% with the

Table 3. In vitro dry matter digestion of 8 substrates inoculated with 5 and 15 ml. of rumen liquor obtained from a fistulated steer.

SUBSTRATE	5 ml.				15 ml.			
	Run 1	Run 2	Run 3	Pooled Means	Run 1	Run 2	Run 3	Pooled Means
Alfalfa Hay								
Mean %	42.55	41.18	41.68	41.80	53.83	50.83	50.03	51.56
S. D. [1] %	2.34	1.02	1.76	1.58	.84	.67	.46	2.05
C. V. [2] %	5.50	2.48	4.22	3.78	1.56	1.32	.92	3.98
Alfalfa (green chop)								
Mean %	52.90	52.88	53.13	52.97	49.95	50.90	50.93	50.59
S. D. %	.41	.73	.53	.86	3.66	2.85	.19	2.28
C. V. %	.78	1.38	1.00	1.62	7.33	5.60	.37	4.51
Meadow Grass Hay 1								
Mean %	50.02	48.90	49.70	49.55	56.55	58.53	53.30	56.13
S. D. %	.49	2.53	.97	1.53	2.12	.88	1.75	2.69
C. V. %	.98	5.17	1.95	3.09	3.75	1.50	3.28	4.79
Meadow Grass Hay 2								
Mean %	40.13	43.03	40.28	41.14	47.00	48.22	48.53	47.92
S. D. %	1.19	.79	.29	1.59	1.29	2.64	1.35	1.60
C. V. %	2.97	1.84	.72	3.86	2.74	4.23	2.78	3.34
Meadow Grass Hay 3								
Mean %	35.15	34.78	35.80	35.24	42.73	44.35	38.95	41.93
S. D. %	.58	2.02	1.16	1.33	2.45	1.05	.90	2.67
C. V. %	1.65	5.81	3.24	3.77	5.73	2.37	2.31	6.37
Meadow Grass Hay 4								
Mean %	34.80	31.70	33.60	33.37	41.70	42.83	36.80	40.44
S. D. %	.37	3.37	.45	2.31	2.12	1.48	3.83	3.64
C. V. %	1.06	10.63	1.34	6.92	5.08	3.46	10.41	9.00
Mixed Grass Hay								
Mean %	41.53	41.35	43.60	42.16	53.05	53.13	49.05	51.74
S. D. %	.71	1.32	1.23	1.47	1.95	.46	.37	2.25
C. V. %	1.71	3.19	2.82	3.49	3.68	.87	.75	4.35
Sagebrush								
Mean %	29.68	31.38	32.98	31.34	35.05	34.58	35.68	35.10
S. D. %	.19	.22	.79	1.47	.61	.17	.19	.52
C. V. %	.64	.70	2.40	4.69	1.74	.49	.53	1.48

[1] Standard deviation.

[2] Coefficient of variation.

Table 4. In vitro dry matter digestion of 8 substrates inoculated with 5 and 15 ml. of rumen liquor obtained from fistulated lambs.

SUBSTRATES	5 ml.				15 ml.			
	Run 1	Run 2	Run 3	Mean	Run 1	Run 2	Run 3	Mean
Alfalfa Hay								
Mean %	49.88	50.78	47.48	49.38	47.90	49.45	47.78	48.38
S. D. %[1]	1.01	.56	.61	1.61	.14	.73	.67	.90
C. V. %[2]	2.02	1.10	1.28	3.26	.29	1.48	1.40	1.86
Alfalfa (green chop)								
Mean %	50.93	51.93	50.58	51.14	48.90	49.83	49.30	49.34
S. D. %	.83	.64	.61	.87	1.64	.47	.38	1.02
C. V. %	1.63	1.23	1.21	1.70	3.35	.94	.77	2.07
Meadow Grass Hay 1								
Mean %	45.53	47.68	44.40	45.87	45.93	49.15	49.65	48.24
S. D. %	1.14	.57	1.10	1.67	2.93	.59	1.33	2.41
C. V. %	2.50	1.20	2.48	3.64	6.38	1.20	2.68	5.00
Meadow Grass Hay 2								
Mean %	40.50	41.88	40.00	40.79	41.65	41.95	42.07	41.89
S. D. %	.62	.70	1.88	1.37	.70	1.64	.36	.97
C. V. %	1.53	1.68	4.70	3.36	1.68	3.91	.86	2.32
Meadow Grass Hay 3								
Mean %	36.75	38.60	37.35	37.57	38.88	38.00	37.33	38.07
S. D. %	.19	2.55	1.10	1.66	.57	.90	1.15	1.05
C. V. %	.52	6.66	2.95	4.42	1.47	2.37	3.08	2.76
Meadow Grass Hay 4								
Mean %	34.53	36.32	35.30	35.38	37.65	36.30	37.23	37.06
S. D. %	.88	.97	.88	1.13	.70	1.04	.73	.97
C. V. %	2.55	2.67	2.49	3.19	1.86	2.87	1.96	2.59
Mixed Grass Hay (OSU)								
Mean %	45.60	48.03	43.78	45.80	46.70	45.80	47.33	46.61
S. D. %	.63	.65	.39	2.69	1.10	.95	1.56	2.75
C. V. %	1.38	1.35	.89	5.87	2.36	2.07	3.30	5.90
Sagebrush								
Mean %	38.23	38.95	34.75	37.31	37.75	37.70	36.43	37.29
S. D. %	.50	.58	.21	1.96	.13	.28	.50	.77
C. V. %	1.31	1.49	.60	5.25	.34	.74	1.37	2.06

[1] Standard deviation.

[2] Coefficient of variation.

following distribution:

Standard Deviations Range (%)	Run Means (% observed)	Pooled Means (% observed)
3.00 - 3.83	3	3
2.00 - 3.00	10	28
1.00 - 2.00	25	46
.50 - 1.00	37	23
.13 - .50	25	--
	100	100

The above variation due to the pooled means is a reflection of both the within and between trial variability. Similar magnitudes of variation for dry matter digestibility in vitro have been previously reported in this laboratory (20, p. 973; 85, p. 26).

Analysis of Variance

Table 5 contains the analysis of variance for the split-split-plot design used in this study and Appendix, Tables 1, 2, 3, 4, and 5 show the appropriate two-way and one-way tables of means. The inclusion of two sources of inoculum, cattle and sheep, was not to compare cattle and sheep effects per se, but rather to evaluate the versatility of the artificial rumen procedure. Other variables studied were the level of rumen liquor, location of bottles in the fermentation flask, and the digestibility of substrates.

Whole-plot. The F-values indicate that no significant differences were observed with either the three replication effects

Table 5. Analysis of variance for the split-split plot design used in standardizing the *in vitro* procedure.

Source of Variation	Degrees of Freedom	Mean Square	F-value
Whole Plot			
Replication	2	41.9477	7.20
Inoculum	1	61.1204	10.49
Whole Plot Error	2	5.8255	
Sub Plot			
Level	1	1003.6267	105.40 **
Substrate	7	1852.4075	194.55 **
Alfalfa (green chop) vs. Grass Hay 1	1	28.17	2.96
Grass Hay 1 vs. Alfalfa	1	112.67	11.83 **
Alfalfa vs. Mixed Grass Hay	1	31.51	3.31
Mixed Grass vs. Grass Hay 2	1	326.34	34.27 **
Grass Hay 2 vs. Grass Hay 3	1	534.04	55.88 **
Grass Hay 3 vs. Grass Hay 4	1	66.67	7.00 *
Grass Hay 4 vs. Sagebrush	1	40.04	4.24 *
Level X Substrate	7	67.1624	7.05 **
Whole Plot X Sub Plot			
Level X Inoculum	1	729.8551	76.65 **
Substrate X Inoculum	7	109.4571	11.50 **
Level X Substrate X Inoculum	7	34.0664	3.58 **
Replication X Level	2	3.5658	.37
Replication X Substrate	14	3.6868	.39
Sub Plot Error	44	9.5218	
Sub Sub Plot			
Location	3	10.5146	8.91 **
L_0 vs. L_1, L_2, and L_3	1	31.27	26.49 **
L_1 vs. L_2 and L_3	1	.25	.21
L_2 vs. L_3	1	.02	.02
Location X Replication	6	1.9246	1.63
Location X Inoculum	3	1.7601	1.49
Location X Level	3	14.6412	12.40 **
Location X Substrate	21	1.9555	1.66 *
Location X Substrate X Level	21	1.9201	1.63 *
Location X Substrate X Inoculum	21	1.4654	1.24
Location X Inoculum X Level	3	7.4661	6.33 **
Location X Inoculum X Level X Substrate	21	1.9004	1.61 *
Sub Sub Plot Error	186	1.1804	
TOTAL	383		

* $P < 0.05$
** $P < 0.01$

or source of inoculum (sheep or cattle). However, both of these factors are confounded because within-species variability could not be measured. Mean dry matter digestibility of 43.94% and 43.14% were obtained with inoculum from cattle and sheep respectively (Appendix, Table 1). Mean digestibilities are 43.56%, 44.10%, and 42.96% for replications 1, 2, and 3 respectively.

Sub-plot. A statistically significant difference ($P<0.01$) was found when rumen liquor was increased from 5 to 15 ml. Alfalfa (green chop) had lower dry matter digestibility at 15 ml than for 5 ml of rumen inoculum (Appendix, Table 2), consequently, a level times substrate interaction was significant ($P<0.01$).

The magnitude of difference in dry matter digestibility resulting from 5 and 15 ml levels of rumen fluid was significantly greater with inoculum from cattle than from sheep ($P<0.01$). The three-way interaction between the level of rumen fluid, source of inoculum, and substrates digested was significant ($P<0.01$), but this type of three-way interaction is difficult to interpret biologically. No interaction between replication and level of rumen fluid was observed.

Mean dry matter digestibility for the substrates is presented in the Appendix, Table 2. As anticipated, differences among the eight substrates were highly significant ($P<0.01$). No significant

difference was found between alfalfa (green chop) and meadow grass hay 1, but both had greater dry matter digestion than any of the other substrates. Likewise, no significant difference was noted between alfalfa hay and the mixed grass hay, but both were more highly digestible than the last three meadow grass hays or the sagebrush. The four meadow grass hays decreased significantly in dry matter digestion with stage of maturity. Sagebrush was not digested at as high a level as any of the other substrates.

Interaction between replications and substrates was not evident. However, a significant interaction was noted between the substrates and source of inoculum (Appendix, Table 3). The trend suggests that inoculum from sheep tended to digest alfalfa hay and sagebrush at a higher rate than inoculum from cattle; conversely, inoculum from cattle tended to digest alfalfa (green chop) and meadow grass hays 1 and 2 to a greater extent than inoculum from sheep. Digestibilities for the mixed grass hay and meadow grass hays 2 and 3 were comparable for both sources of inoculum.

Sub-sub-plot. Significant differences were noted between the number one location and the other three positions of the fermentation flasks. Two postulates to explain the reduced dry matter digestibility in the number one position are as follows: (1) cold

carbon dioxide may have decreased the temperature of the first flask; and (2) loss of volatile fatty acids, methane, and other end products may be greater since there is no carry-over into the number one flask. The location effect could cause experimental error if the substrates did not have an equal opportunity of occupying each of the four positions; therefore, a precautionary step was taken to place all substrates investigated in each of the four positions.

No significant interactions were observed between either the four locations and three replications or the locations and source of inoculum. With the 5 ml level of rumen fluid, the number one position had higher digestibility than the fourth position, but the 15 ml level of rumen liquor only showed a difference between the first position and the last three locations (Appendix, Table 4). The significant interaction ($P<0.05$) observed between locations and substrates is difficult to explain. From the percent dry matter digestibility in Appendix, Table 5, it appears that all substrates had higher digestibilities in the last three positions except the two alfalfa samples. Alfalfa hay had greater digestibility in the first position than either the second or third locations, and alfalfa (green chop) had higher dry matter digestibility in the first location than in the last position. For practical purposes this interaction would need more exploration to substantiate its occurrence.

Results from this analysis indicate that forages of varying nutritional value may be differentiated with in vitro dry matter digestibility. Furthermore, a procedure which is repeatable has been used with inoculum from both sheep and cattle. Increasing the level of rumen liquor from 5 to 15 ml resulted in greater dry matter digestibility in vitro, but to a greater degree with inoculum from cattle than from sheep.

The main limitation to the procedure was the effect of fermentation position on in vitro dry matter digestibility. Therefore, it is suggested that substrates should be fermented in a series of four whenever comparisons are attempted. This precaution was adhered to in the subsequent artificial rumen studies.

Correlations and Prediction Equations

Bowden and Church (21, p. 980-985) have summarized several significant correlations between the in vitro DM digestibilities of forages and both their in vivo digestibility and chemical composition. The purpose in obtaining the correlations in this study was to develop working prediction equations from the in vitro DM digestibilities of forages.

The in vivo digestibilities for the nine substrates used for correlations are given in Table 1 and the chemical composition in

Table 2. Percent in vitro dry matter digestibility is shown in Table 7 with means from 12 observations for all substrates except the fescue for which only eight were obtained.

Correlations Among In Vivo Digestibilities

Correlation coefficients in Table 6 indicate that in vivo dry matter digestibility is closely related to both in vivo organic matter digestibility (r = .9927) and in vivo energy digestibility (r = .9828). The correlation coefficient (r = .6373) between in vivo dry matter digestibility and in vivo crude protein digestibility is also approaching significance. In general, these correlations show a close association among in vivo digestion coefficients for dry matter, energy, organic matter, and crude protein. Consequently, a reliable method for predicting the digestibility of any one of these items should also apply to the other items.

Correlations Between In Vitro Dry Matter Digestibility and In Vivo Digestibilities

Correlation coefficients in Table 8 were obtained from the in vitro DM digestibilities (Table 7) and the in vivo digestibilities in Table 1. With 15 ml of inoculum from sheep, correlations of in vitro dry matter digestibility with in vivo dry matter digestibility (r = .9779), in vitro dry matter digestibility with in vivo organic

Table 6. Correlations between all possible combinations of *in vivo* coefficients.

Factors Correlated	Correlations
In vivo dry matter digestibility with:	
In vivo crude protein digestibility	.6373
In vivo cellulose digestibility	.4673
In vivo organic matter digestibility	.9927 **
In vivo energy digestibility	.9828 **
In vivo crude protein digestibility with:	
In vivo cellulose digestibility	.5852
In vivo organic matter digestibility	.6701 *
In vivo energy digestibility	.7512 *
In vivo cellulose digestibility with:	
In vivo organic matter digestibility	.5343
In vivo energy digestibility	.5467
In vivo organic matter digestibility with:	
In vivo energy digestibility	.9925 **

* $P < 0.05$
** $P < 0.01$

Table 7. Summary of *in vitro* dry matter digestibility of the 9 substrates used for correlations.

Substrate	Mean In Vitro DM Digestibility[1]			
	Inoculum (Cattle)		Inoculum (Sheep)	
	5 ml	15 ml	5 ml	15 ml
Alfalfa Hay	41.80 ± 1.58[2]	51.56 ± 2.05	49.38 ± 1.61	48.38 ± .90
Alfalfa (green chop)	52.97 ± .86	50.59 ± 2.28	51.14 ± .87	49.34 ± 1.02
Meadow Grass Hay 1	49.55 ± 1.53	56.13 ± 2.69	45.87 ± 1.67	48.24 ± 2.41
Meadow Grass Hay 2	41.14 ± 1.59	47.92 ± 1.60	40.97 ± 1.37	41.89 ± .97
Meadow Grass Hay 3	35.24 ± 1.33	41.93 ± 2.67	37.57 ± 1.66	38.07 ± 1.05
Meadow Grass Hay 4	34.80 ± 3.37	40.44 ± 3.64	35.38 ± 1.33	37.06 ± .97
Fescue	53.03 ± 5.54	73.05 ± .78	64.65 ± 2.02	64.87 ± 1.76
Mixed Grass Hay	41.53 ± .71	51.74 ± 2.55	45.80 ± 2.69	46.61 ± 2.75
Sagebrush	29.68 ± .19	35.10 ± .52	37.31 ± 1.96	37.29 ± .77

1. Eight observations for fescue; 12 for other substrates.
2. Standard deviation.

Table 8. Correlations of in vitro DM digestibility, at 5 and 15 ml levels of rumen fluid with inoculum from sheep and cattle, and in vivo digestion coefficients from 9 substrates.

Factors Correlated	Correlations	Regression Equation
In vitro DM digestibility (5 ml cattle) with:		
In vivo dry matter digestibility	.8444 **	24.2 + .8322 X
In vivo crude protein digestibility	.8535 **	-16.6 + 1.7152 X
In vivo organic matter digestibility	.8797 **	21.6 + .9408 X
In vivo energy digestibility	.9061 **	17.9 + .9577 X
In vivo cellulose digestibility	.7079 *	
In vitro DM digestibility (5 ml sheep) with:		
In vivo dry matter digestibility	.9677 **	20.8 + .8516 X
In vivo crude protein digestibility	.7385 *	- 4.0 + 1.3242 X
In vivo organic matter digestibility	.9713 **	19.4 + .9280 X
In vivo energy digestibility	.9845 **	16.3 + .9293 X
In vivo cellulose digestibility	.4448	
In vitro DM digestibility (15 ml cattle) with:		
In vivo dry matter digestibility	.9402 **	25.0 + .6906 X
In vivo crude protein digestibility	.6807 *	5.3 + 1.0185 X
In vivo organic matter digestibility	.9377 **	24.2 + .7469 X
In vivo energy digestibility	.9371 **	21.7 + .7370 X
In vivo cellulose digestibility	.5417	
In vitro DM digestibility (15 ml sheep) with:		
In vivo dry matter digestibility	.9779 **	18.2 + .8988 X
In vivo crude protein digestibility	.7025 *	- 4.3 + 1.3172 X
In vivo organic matter digestibility	.9762 **	16.6 + .9734 X
In vivo energy digestibility	.9810 **	14.1 + .9673 X
In vivo cellulose digestibility	.4298	

* $P < 0.05$

** $P < 0.01$

matter digestibility (r = . 9762), and in vitro dry matter digestibility with in vivo energy digestibility were consistently higher than corresponding correlations with the three other in vitro digestibilities. However, all four in vitro dry matter digestibilities were significantly correlated ($P<0.01$) with in vivo digestion of dry matter, organic matter, and energy.

With 5 ml of inoculum from cattle, in vitro dry matter digestibility was significantly correlated with in vivo digestibility of crude protein ($P<0.01$) and cellulose ($P<0.05$). In vitro dry matter digestibility (15 ml inoculum from cattle, 5 and 15 ml inoculum from sheep) was significantly correlated with in vivo digestibility of crude protein ($P<0.05$), but cellulose correlations were not significant for the limited comparisons made in this study. However, a significant correlation coefficient (r =.95) between in vitro dry matter digestibility and in vivo cellulose digestibility has been reported in this laboratory by Bowden and Church (21, p. 982).

Magnitudes of correlation coefficients in Table 8 indicate that the in vitro fermentation procedure used in this study has a definite value in estimating the relative nutritional value of forages.

Correlations Between In Vivo Digestibility and Chemical Composition

For the substrates used, the only significant correlations

observed were between in vivo digestible crude protein and both percent crude protein and percent ash (Table 9). There appears to be a trend towards relationship between percent crude protein in vivo digestibility of dry matter, energy, organic matter, and cellulose. Conversely, percent ether extract, percent crude fiber, and percent cellulose appear to have a negative association with in vivo crude protein digestibility, in vivo dry matter digestibility, and in vivo digestible energy.

Many attempts have been made to correlate a certain chemically determined nutrient with the nutritive value of forages. In most instances, these correlations have not been very satisfactory (14, p. 62-68).

Correlation Between In Vitro Digestibilities and Chemical Composition

Generally, low correlation coefficients in Table 10 were obtained for in vitro dry matter digestibility with the chemical constituents of the substrates. Since limited comparisons were made, it is entirely possible that a consistent relationship may exist between in vitro dry matter digestibility and percent crude protein. A significant correlation ($P < 0.05$) was obtained for in

Table 9. Correlations between in vivo digestibility coefficients for the 9 substrates and their corresponding chemical compositions.

Chemical Identity (%)	In vivo Digestibility Coefficients				
	DM	DCP	DE	DOM	DC
Dry Matter	-.1098	.2100	-.0576	-.1334	.0255
Crude Protein	.4387	.8177**	.5676	.4846	.3852
Crude Fiber	-.3976	-.3814	-.4368	-.4098	-.0734
Ether Extract	-.2725	-.5554	-.3486	-.3011	.0018
Cellulose	-.3135	-.4378	-.3713	.0574	-.0885
Organic Matter	.2017	.5025	.0311	.1059	.4823
Ash	.1185	.7605*	.2904	.1897	.4964

* $P < 0.05$
** $P < 0.01$

Table 10. Correlations between in vitro dry matter digestibility using 5 and 15 ml levels of rumen fluid with inoculum from sheep and cattle, and the chemical composition of 9 substrates.

Chemical Identity (%)	In vitro DM Digestibility			
	5 ml cattle	5 ml sheep	15 ml cattle	15 ml sheep
Dry Matter	-.1544	.0427	-.1694	.0427
Crude Protein	.6754*	.5649	.3355	.4771
Ether Extract	-.3879	-.3810	-.5334	-.4070
Crude Fiber	-.4310	-.4330	-.1634	-.3583
Cellulose	-.4228	-.3581	-.2870	-.2874
Organic Matter	-.3150	.0777	.0704	.1126
Ash	.4685	.3202	.2169	.2525

* $P < 0.05$

in vitro dry matter digestibility (5 ml of inoculum from cattle) with percent crude protein (r = .6754), which is in agreement with an earlier report (21, p. 982). Percent crude fiber, cellulose, and ether extract appear to have a negative association with *in vitro* dry matter digestibility. Bowden and Church (21, p. 982) reported a significant correlation (r = .41) between percent cellulose and *in vitro* dry matter digestion; only one plant species (fescue) and a larger sample size (39) were included in their work. However, correlations of this nature account for such a small amount of the total variability that they are probably not useful for the prediction of forage value.

Predicting the Nutritive Value

The efficacy of the previously described artificial rumen procedure, utilizing 15 ml of inoculum from sheep, as a predictor of forage energy digestibility was tested using 12 additional samples of known energy digestibility. To test the *in vitro* procedure, especially the regression equations in Table 8, it seemed advisable to use forages other than those used to develop the relationships. The mean *in vitro* dry matter digestibilities, calculated *in vivo* energy digestibilities, and actual *in vivo* energy digestibilities are shown in Table 11. No significant difference (t = .0396) was found

when the calculated and actual energy digestibilities were analyzed by the Student's t-test (77, p. 132).

The author does not intend to imply that this analysis is all-inclusive for evaluating the prediction equation, but rather a preliminary confirmation of the equation. Certainly, additional substrates should be digested in vitro to increase the reliability of the prediction equations. It does appear that these equations are adequate for differentiating between substrates of varying nutritional value. Therefore, the equations will be used to compare sagebrush samples in the next section.

Table 11. Calculated DE, from equations developed for DM digestibility in vitro using 15 ml of inoculum from sheep for 12 samples of known digestible energy.

SUBSTRATE	(1) In Vitro DM Digestibility(X)	(2)[2] Calculated In Vivo DE(Y)	(3) Actual In Vivo DE	Difference[3] (2) - (3)
Light RGS	34.22±[1] .97	47.2	51.8	-4.6
Heavy RGS	63.07± 1.20	75.1	68.3	6.8
Alfalfa(OSU)	43.20± .93	55.9	56.4	-0.5
Alfalfa(Can.)	49.75± 1.03	62.2	60.0	2.2
Bromegrass(Can)	40.22± 1.44	53.0	54.4	-1.4
Fescue 228-1	58.42± .30	70.6	70.7	0.1
Fescue 228-2	58.00± .88	70.2	71.0	-0.8
Fescue 228-3	56.77± .62	69.0	67.4	1.6
Fescue 232-2	58.80± .66	71.0	69.8	1.2
Fescue 232-3	64.28± .44	76.3	70.7	5.6
Fescue 232-5	46.73± .22	59.3	63.0	-3.7
Fescue 215.2	56.37± .74	68.6	69.6	-1.0

[1]Standard deviation
[2]Equation: Y = 14.1 + .9673X (See Table 8)
[3]t = .0396

Part II. NUTRITIONAL VALUES OF SAGEBRUSH

REVIEW OF LITERATURE

Approximately 422, 275 square miles of land support sagebrush growth in the Western United States (15, p. 12), while some 300 species of the plant genus, Artemesia, are distributed throughout the temperate regions of the world (15, p. 19). Beetle in 1960 (15, p. 36) named big sagebrush, Artemesia tridentata tridentata or typica, and the sagebrush referred to throughout this thesis belongs to this species. Oregon has about 22, 000 square miles of land supporting the growth of sagebrush (15, p. 13); consequently it is economically sound to utilize the nutrients supplied by sagebrush for both game and livestock production.

Evidence is available to indicate that deer and other game utilize appreciable quantities of sagebrush when ingested along with other plants. However further evidence indicates that deer cannot exist on sagebrush alone. The essential oil content of sagebrush is relatively high and may be a factor which adversely affects utilization.

In Vivo Effects of Sagebrush

Animal digestibility trials (37, p. 590; 101, p. 289; 102, p.

8-13; 18, p. 78) have not shown any toxic effects from sagebrush. The specific chemical activity of the essential oils in sagebrush has not been ascertained; however, indirect methods have been undertaken to study the effects of essential oils. Pure culture plates have shown specific microorganism susceptibility to essential oils (82, p. 378-381).

Digestibility Trials

Cook and associates in 1952 (37, p. 590) and Cook, Stoddart, and Harris (38, p. 26) found that big sagebrush had high gross energy and total digestible nutrient values, but was relatively low in metabolizable energy. It was also noted that essential oils were eliminated with the urine, and did not represent available energy to sheep. Big sagebrush was the least palatable of several browse species studied; and due to the low intake, the energy furnished by the sagebrush was below recommended standards (38, p. 25).

Smith (101, p. 285-289; 102, p. 8-13) obtained low consumption of sagebrush by deer in digestion trials, and the deer consistently lost weight. However, mixed diets containing sagebrush markedly increased the intake of each ingredient. This Utah worker concluded that sagebrush may have a high value in a mixed diet, but may be inadequate as the sole source of forage (102, p. 13).

Digestibility coefficients of sagebrush were about one half as much for protein and twice as high for ether extract and carbohydrates as in the case of green alfalfa (101, p. 289).

Bissell et al. (18, p. 57-78), in a similar study to that of Smith (101, p. 285-289), found that sagebrush was highly unpalatable to mule deer. These California workers reported values of 55 percent for TDN and 41 percent for digestible protein. No digestive disturbances were noted with the possible exception of one deer with diarrhea on the sagebrush experiment (18, p. 75).

In a recent publication in 1962 Dietz and associates (51, p. 1-81) summarized the deer digestibility data that they had reported earlier in Colorado studies (48, p. 6-10; 49, p. 4-46; 52, p. 151-158). Mean digestible nutrient values for sagebrush were as follows: digestible protein, 52.53%; crude fat, 76.54%; crude fiber, 39.53%; nitrogen-free extract, 52.11%; and TDN, 58.94% (51, p. 69).

Dietz, Udall, and Yeager (51, p. 76) have summarized the digestion coefficients for sagebrush and alfalfa that were obtained by Smith (101, p. 285-289) and Bissell (51, p. 57-78). It is apparent that digestion coefficients for sagebrush are quite comparable to those for alfalfa. Conversely, consumption was always subnormal on complete sagebrush diets, which suggests a palatability problem.

Essential Oils

Bissell et al. (18, p. 57-78) suggested that sagebrush oil may have a potential deleterious action on the rumen microorganisms. Earlier work in 1946 by Carlson and associates (28, p. 155-168) demonstrated that saline extracts of sagebrush possessed anesthetic properties when injected into chickens and mice. These saline extracts were also found to have antibacterial and antimalarial activity, but their specific chemical activity was not ascertained (28, p. 168).

Maruzella and Lichtenstein in 1956 (82, p. 378-381) studied the effect of volatile oils from sagebrush on the inhibition of the following microorganisms: Neisseria perflava, Sarcina lutea, Bacillus mesentericus, Aerobacter aerogenes, Bacillus subtilis, Micrococcus pyogenes, Pseudomonas aeruginosa, Serratia marcescens, Escherichia coli, and Proteus vulgaris. Inhibition from sagebrush oils was demonstrated only for Sarcina lutea, Aerobacter aerogenes, and Serratia marcescens.

The antifungal activity of 92 volatile oils was tested with 18 organisms both pathogenic and nonpathogenic (83, p. 250-254). Sagebrush oil was not included in this study, but volatile oils from white cedar did exhibit fungicidal as well as bacteriocidal effects.

Recent work by Short in 1963 (100, p. 192) showed that microorganisms from the rumen of a steer were adversely affected by certain chemical components of white cedar; conversely microorganisms from the deer rumen were not adversely affected by the inhibitory action of oils from white cedar. These observations were obtained from fermentations in an artificial rumen.

The implications from this literature are that the volatile oils may have the following effects on microorganisms: anesthetic, bactericidal, bacteriostatic, fungicidal, and stimulatory.

Chemical Analyses of Sagebrush

Wilson et al. in 1906 (112, p. 1-41) published chemical analyses of big sagebrush, which included values for ash, crude fat, crude fiber, crude protein, and nitrogen-free extract. More recently, Hamilton (63, p. 1-44) in a comprehensive study published 66 analyses of sagebrush obtained on various dates and localities in Wyoming. In addition to proximate analysis, he also included determinations for calcium, phosphorus, magnesium, manganese, and carotene. Dietz (47, p. 1-37) and Dietz et al. (50, p. 118-122) have studied the seasonal effects on the chemical constituents of sagebrush at different sites in Colorado. When compared to several other browse species, big sagebrush had the highest seasonal

mean percentage of protein, phosphorus, crude fat, and moisture; however, it had the lowest mean percentages of crude fiber, nitrogen free extract and calcium. Big sagebrush had its highest percentages of important nutrients during the winter (50, p. 118-122).

Cook and Harris (38, p. 43) found that sites indirectly affect the chemical content of plants and plant parts through soil and plant development, water runoff, intensity of shade, and other environmental factors. They concluded further that environmental factors including soil moisture are more important in determining the nutrient content of range forage plants than is the chemical content of the soil.

Methods for planting, cultivating and harvesting sagebrush have been extensively outlined (61, p. 719). It is also suggested to apply 5-10-5 commercial fertilizer at the rate of 700 pounds to the acre for maximum yields. Sagebrush will withstand average winter conditions, 15° F. below zero, but will be subjected to winterkill when temperatures remain below 15° F. for extended periods (61, p. 721).

From the above reports, it is suggested that sagebrush contains adequate amounts of valuable nutrients, which make it a "potential" key browse species for deer and sheep during the critical winter season.

Kinney et al. (75, p. 290-294) found no quinine present in sagebrush; the bitter taste was attributed to the presence of glucosides. This group also fractionated tripalmitins, waxes, and soluble sugars from big sagebrush.

The steam volatile oils of sagebrush are liquid with a powerful, camphoraceous, stinging and lachrymatory odor (62, p. 429). Adams and Billinghurst (1, p. 2895-2903) found that the maximum yields of sagebrush oils occurred in the late summer or in the fall; moreover the oil was most easily removed from material that was air dried prior to steam distillation. Essential oil yields obtained from leaves and young shoots have been about one percent of dry matter (1, p. 2895-2903; 99, p. 457-459). Kinney et al. (75, p. 612-625) reported yields of oil ranging from 0.45 percent from fresh plant material to 1.26 percent from dried material. Bissell et al. (18, p. 70) obtained 12 percent volatile oils from sagebrush, which is a considerably greater yield than has been reported by other research workers.

Adams and Oakberg in 1934 (99, p. 459) were the first to characterize the volatile oils of sagebrush; these oils were artemisol 5%, α-pinene 20%, cineole 7%, l-camphor 40%, sesquiterpenes 12% and resins 16%. Subsequently, in 1941 Kinney et al. (75, p. 612-625) identified the following oils in sagebrush: α-pinene 20.7%,

cineole 28.8%, camphor 25.3%, artemisol 14.4%, and lost residue 11.3%.

Part II. NUTRITIONAL VALUES OF SAGEBRUSH

EXPERIMENTAL PROCEDURE

The literature review has included several items of interest pertaining to the nutritional effects of sagebrush. Information has been scanty however, on the detection of possible deleterious responses from big sagebrush which is of concern in this study. Specific areas of investigation are as follows: (1) *in vivo* digestibility of sagebrush and (2) evaluation of the effects of fertilization on monthly sagebrush clippings.

In Vivo Digestibility of Sagebrush

The experimental design used in the digestion trials is shown in Table 12. Prolonged preliminary feeding periods were necessary since the lambs did not relish sagebrush. The percent of sagebrush in the mixtures is the maximum amount that the lambs would consume in a given mixture. In Trial 3, sagebrush was forced into the rumen through the fistula of two wether lambs.

Table 12. In Vivo Experimental Design.

Substrate	Number of Lambs	Trial
Alfalfa Hay (chopped)	2	I
Mixture	2	
78% Alfalfa Hay (chopped)		
22% Sagebrush (chopped)		
Alfalfa (green-chopped)	4	II
Mixture	4	
61% Alfalfa Hay (green chopped)		
39% Sagebrush (chopped)		
Mixed Grass Hay	4	
Mixture	2	
86% Mixed Grass Hay		
14% Sagebrush (chopped)		
Alfalfa Hay (pelleted)	4	III
Mixture	2	
51% Alfalfa Hay (pelleted)		
49% Sagebrush (ground)		

Substrates

Sagebrush. The collection of sagebrush was a very laborious job; common hedge shears were used to clip the annual growth. Following clipping, the samples were bagged in plastic, transported to Corvallis, run through an ensilage chopper and frozen. Sagebrush fed in Trial 1 was collected near Silver Lake, Oregon, and that fed in Trials 2 and 3 was collected at Bend, Oregon. Chopped frozen sagebrush was fed in Trials 1 and 2, while frozen sagebrush was ground in a Wiley mill (3/16 inch mesh) for use in Trial 3.

Alfalfa Hay. Baled alfalfa hay fed in Trial 1 was chopped in an ensilage chopper before it was fed. The alfalfa fed in Trial 2 was field-chopped when about 18 inches high and immedicately frozen; hereafter, it will be referred to as green-chop alfalfa. Pelleted alfalfa hay (10% molasses) was fed in Trial 3.

Mixed Grass Hay. A straw-like, mixed grass hay consisting primarily of Meadow Fescue (Festuca elatior) and Perennial Ryegrass (Lolium perenne) was fed in Trial 2.

Feeding Trials

Crossbred wether lambs weighing 75-100 pounds were obtained, and maintained in metabolism stalls modified from the design of Briggs and Gallup (23, p. 480). These stalls (Figure 5) allow complete separation of the urine and feces, and provide access to water, feed, steamed bone meal, and salt. The lambs were fed twice daily; urine and feces were collected, and weights were recorded each evening during the 7-day collection period, following a fifteen day preliminary feeding period. In Trial 1 Hydrochloric acid was added to the urine to reduce bacterial action until it could be refrigerated; whereas, in the subsequent trials the urinalysis was not included. The feces were weighed, frozen until the trial was completed, transferred to an oven for drying, then ground for

Figure 5. Two fistulated lambs in a digestion crate designed after that of Briggs and Gallup (23, p. 479).

Figure 6. Forced feeding of sagebrush. Note the funnel by which sagebrush is forced into the rumen of a fistulated lamb.

chemical analysis.

In Trial 3 pelleted alfalfa hay (10% molasses) was fed to two fistulated wether lambs; in addition ground (3/16 inch mesh) sagebrush was forced into the rumen via the fistulas (Figure 6). Otherwise, processing of feed and feces was the same as that used in the other digestion trials.

Chemical Analyses of Feed and Feces

The methods for the chemical analyses of feed, feces, and urine have been previously described in this section (Chemical Composition). Table 13 presents the chemical analyses of sagebrush, alfalfa hay (Trials 1 and 2), alfalfa (green chop), and the mixed grass hay. In Trials 1 and 3, sagebrush was fed with alfalfa hay, which is a good source of crude protein, but contains lower amounts of ether extract than sagebrush. Sagebrush also had higher values for NFE than these alfalfa hays, but it had less crude fiber and ash. In Trial 2, sagebrush was fed with a succulent, high protein alfalfa (green chop), and in another combination it was fed with a low protein mixed grass hay. Alfalfa (green chop) was low in crude fiber, cellulose, and NFE, but quite high in crude fat and ash. The mixed grass hay was high in crude fiber and cellulose.

Table 13. Chemical Composition of the Feeds, Percent[1]

	Crude Protein	Crude Fiber	Ether Extract	Ash	NFE	Cellulose	Gross[2] Energy
Trial I							
Alfalfa Hay (chopped)	15.5	27.5	2.4	7.1	47.5	30.9	4.47
Sagebrush	8.6	22.7	7.9	4.7	56.1	27.3	4.94
Trial II							
Alfalfa (green chop)	24.0	19.5	3.0	11.5	42.0	23.5	4.46
Mixed Grass Hay	6.5	33.8	2.6	6.2	50.9	34.6	4.35
Sagebrush	8.5	22.8	7.0	4.5	57.2	29.0	4.88
Trial III							
Alfalfa Hay (pelleted)	16.2	28.4	1.6	10.3	43.5	27.6	4.20
Sagebrush	8.5	22.8	7.0	4.5	57.2	29.0	4.88

[1] Dry matter basis.
[2] Kilocalories per gram.

Evaluation of Fertilized Plots of Sagebrush

Near Silver Lake, Oregon, three collection plots were established to study the effect of season and rate of fertilization on the chemical composition and in vitro digestibility of sagebrush. The three plots are as follows: (1) plot one was on a deep soil site; (2) plot two was on a hillside; and (3) plot three was near the forest. One half of each plot was fertilized in November 1961 with 400 pounds per acre of a 16-20 ammonium phosphate fertilizer.

In Vitro Digestibility of Sagebrush

The artificial rumen procedure developed in first section of this study was employed to compare the monthly sagebrush samples,

and to study the effects of added essential oils. Basically, the procedure used included the following: (1) 15 ml of rumen fluid from sheep, (2) a 24-hour fermentation period, (3) a standard buffer, and (4) one gram of substrate with four replications per sample. In addition, a standard alfalfa hay was always included as the control, and percent digestibility of sagebrush was always corrected to this standard control. Regression equations from standardization procedures were used to predict the percentage in vivo digestible energy, digestible dry matter, and digestible protein for each of the sagebrush samples.

Steam volatile essential oils were extracted with a steam distillation apparatus. The procedure included boiling the sagebrush in water which freed the steam volatile oils; these in turn were condensed and collected in a water trap. The volume of oil was recorded for each sample, and then Na_2SO_4 was added to remove any remaining water. The oils were pipetted into 10 ml capsules, sealed, and refrigerated prior to their use in the artificial rumen. These oils were added to several substrates in the artificial rumen using a microsyringe to measure 30, 50, and 100 microliters of the oils.

Chemical Composition

Each month a representative one-pound sample of the foliage

was clipped from each fertilized and unfertilized plot. The samples were stored at -10°F, then freeze-dried, and ground through a 40 mesh screen for further analyses. The following chemical determinations were made on each sample from the first two sites: crude protein, ether extract, crude fiber, nitrogen-free extract, dry matter, ash, cellulose, energy, calcium and phosphorus. The quantity of steam distilled volatile oils was measured on sagebrush samples which were collected at several intervals from the forested site.

Percent crude protein, ether extract, crude fiber, nitrogen-free extract, dry matter, and ash were obtained according to the methods outlined by the Association of Official Agricultural Chemists (4, p. 367-373). Cellulose was determined with a modification of the procedure of Crampton and Maynard (42, p. 383-395). Centrifuge tubes containing a .5 gram of substrate and 20 ml of Crampton-Maynard reagent were boiled in a water bath for 30 minutes instead of refluxing. Then 20 ml of ethyl alcohol were added to each flask and then allowed to set for 20 minutes before filtering the residue into a Gooch crucible. Percent cellulose was obtained by noting the weight change in the dry crucibles after the cellulose had burned in a muffle furnace (600° C) for two hours. Gross energy determinations were made with a Parr oxygen bomb calorimeter. Calcium was determined by the procedure of Hemingway (65, p. 164-168), and

phosphorus was determined using a modification of the calorimetric determination of Roger (96, p. 1050). This modification entailed dry ashing, which replaced the wet ashing, and then the ash was taken up in 25 ml of .1N HCl.

Winter Use of Sagebrush

Twenty five randomly selected plants in each fertilized and unfertilized plot were measured and tagged each fall (1961-1962; 1962-1963) then measured again in the spring to determine winter use by deer. The percentage decrease in twig length was used to express winter use, and to compare the use by deer of fertilized and unfertilized plots.

Part II. NUTRITIONAL VALUES OF SAGEBRUSH

RESULTS AND DISCUSSION

In Vivo Digestibility Trials

Three digestibility trials were completed to determine the apparent digestion coefficients of sagebrush. Since the sheep would not consume any appreciable amount of pure sagebrush, all of the data for sagebrush were determined by difference with various forages. Palatability was a constant problem in these trials.

Consumption of Substrates

The consumption data presented in Table 14 represent the maximum amount of sagebrush that the lambs would consume with each of the substrates (Trial 1 and 2). Sagebrush was consumed in greater amounts with the succulent alfalfa (green chop), 306 grams per day, than with either the alfalfa hay, 198 grams per day, or the mixed grass hay, 78 grams per day. This fact implies that the lambs were not necessarily eating the sagebrush to meet their nutrient requirements since the mixed grass hay was of poorer quality than the alfalfa hays. The author would like to suggest the following possibilities for the above responses: (1) the frozen, alfalfa (green chop) containing 80% moisture may have been diluting the unsavory

sagebrush whereas the dry alfalfa and grass hays did not dilute the distasteful sagebrush; (2) the moisture content of the alfalfa (green chop) may have diluted the essential oils in the rumen so that any deleterious effects to microorganisms was reduced; and (3) the alfalfa may contain organic or inorganic nutrients which tend to counteract the undesirable effects of sagebrush.

Table 14. Daily Consumption in Grams of Feed, Oven-Dry Basis.

Trial	Consumption (grams/day, oven-dry basis) Sagebrush	Other Substrates	Bodyweight Change
Trial I		Alfalfa Hay (ground)	
Lamb 1		676	1.0
Lamb 2		946	1.5
Lamb 3	193	698	2.0
Lamb 4	198	709	1.0
Trial II		Alfalfa (green chop)	
Lamb 1		649	2.25
Lamb 2		734	2.50
Lamb 3		752	3.75
Lamb 4		752	1.25
Lamb 5	299	548	2.25
Lamb 6	256	548	3.75
Lamb 7	306	548	2.25
Lamb 8	298	548	3.50
		Mixed Grass Hay	
Lamb 9		495	-1.25
Lamb 10		474	1.50
Lamb 11		578	-.25
Lamb 12		481	1.00
Lamb 13	75	493	-.25
Lamb 14	78	493	.50
Trial III		Alfalfa Hay (pelleted)	
Lambs 1, 2, 3, 4		891	2.50
Lamb 5	415	534	-1.00
Lamb 6	415	346	-2.00

Consumption of sagebrush by wether lambs varied from 75 to 306 grams per day in this study. In a Utah study (36, p. 26), 2.79 pounds per day of pure sagebrush was consumed by mature sheep. Dietz and associates (51, p. 60-78) reported intakes ranging from 201-297 grams per day of sagebrush when fed in combination with one-half pound of alfalfa pellets. Bissel et al. (18, p. 69) obtained an average daily intake of only 98 grams for deer. In 1950 Smith (102, p. 288) reported intakes for pure sagebrush of .98 - 2.14 pounds per hundredweight. The latter three studies were conducted in digestion crates with mule deer, whereas, the Utah study included mature sheep grazing "pure stands" of sagebrush.

The body weight changes in Table 14 do not suggest adverse effects from sagebrush since the lambs on mixed sagebrush rations responded similarly to their controls. The data further show a substantial increase in body weight for the lambs on the sagebrush and alfalfa (green chop) mixture.

Digestion Coefficients

In the Appendix, Table 8, the individual lamb coefficients of apparent digestibility are given for each of the three digestion trials conducted, and the mean digestibility coefficients for each substrate have been grouped together in Table 15. Digestion coefficients were determined for dry matter (DDM),

Table 15. *In Vivo* Mean Digestion Coefficients with Experimental Rations.

Substrates	No. Lambs	Digestion Coefficients [a], Percent: Dry Matter	Crude Protein	Ether Extract	Crude Fiber	Digestible Energy	Cellulose	Nitrogen-Free Extract
Trial I								
Alfalfa Hay (chopped)	2	56.2 ± 3.0	66.8 ± 2.3	34.1 ± 11.2	34.6 ± 2.4	55.0 ± 2.7	56.7 ± 2.2	67.4 ± 1.8
+ 22% Sagebrush	2	55.7 ± 0.0(53.9)	62.4 ± 0.4(32.2)	51.2 ± 0.3(70.5)	37.2 ± 1.6(49.4)	53.9 ± 0.3(50.3)	54.3 ± 0.1(44.0)	64.1 ± 0.0(54.3)
Trial II								
Alfalfa (green-chop)	4	62.7 ± 0.9	77.7 ± 0.5		52.0 ± 2.7	65.1 ± 0.5	70.1 ± 1.5	72.5 ± 1.1
+ 35% Sagebrush	4	60.5 ± 2.6(56.4)	74.6 ± 2.8(59.0)	46.8	43.7 ± 6.7(30.8)	61.8 ± 3.8(56.3)	64.6 ± 4.0(56.3)	67.6 ± 2.5(60.9)
Trial III								
Mixed Grass Hay	4	62.6 ± 1.3	45.8 ± 4.0	65.9 ± 0.3	62.7 ± 2.2	60.5 ± 1.3	69.0 ± 1.1	68.6 ± 0.8
+ 13% Sagebrush	2	61.2 ± 0.7(52.0)	49.4 ± 3.1(67.9)	69.9 ± 2.7(79.2)	57.8 ± 0.8(12.0)	59.4 ± 1.4(53.9)	66.4 ± 1.7(46.7)	67.6 ± 1.5(61.4)
Alfalfa Hay (pelleted)	4	59.5 ± 2.1	72.3 ± 3.5	37.0 ± 4.7	40.8 ± 3.8	59.8 ± 2.1	55.0 ± 3.7	70.5 ± 1.3
+ 49% Sagebrush	2	56.6 ± 2.0(53.9)	65.5 ± 3.1(51.9)	61.2 ± 0.1(67.3)	33.4 ± 0.9(22.7)	54.2 ± 0.5(48.6)	56.2 ± 1.8(57.9)	64.6 ± 3.2 (60.3)
Mean for Sagebrush	10	54.5 ± 1.9	54.0 ± 13.4	72.3 ± 6.2	29.1 ± 13.7	53.1 ± 3.5	52.2 ± 6.4	59.6 ± 3.0

[a] means ± standard deviation; data in ( ) are digestion coefficients for sagebrush, figured by difference.

(EE), crude fiber (DCF), cellulose (DC), energy (DE) and nitrogen-free extract (NFE) in all trials.

In addition to digestion coefficients, metabolizable energy (ME) as defined by Cook and associates was included in the first trial (Appendix, Table 8a). ME values, expressed as kilocalories per kilogram intake (32, p. 579-590), for the alfalfa hay-sagebrush mixture (2025) and for the pure alfalfa hay (2134) were comparable. This finding is in contrast to an earlier report from Utah (32, p. 579-590) in which ME values of only 1130 kilocalories per kilogram intake were reported for sagebrush. The Utah data were collected during the winter when the sheep were on pure stands of sagebrush. Sagebrush was fed in the present study only in mixtures which may have resulted in better utilization of the sagebrush.

Dry matter digestibility. Sagebrush had a mean DDM of 54.5% for the three trials which was quite consistent (1.9% standard deviation) between trials (Table 15). This mean *in vivo* DDM as determined by difference was somewhat less than that for any of the alfalfa hays or the mixed grass hay. However, the DDM (56.4%) for the sagebrush and alfalfa (green chop) mixture was about the same as the DDM (56.2%) for the pure alfalfa hay (chopped). This mean DDM (54.5%) for sagebrush is considerably greater than the DDM (37.6%) reported in 1954 by Cook and associates (38, p. 26) at Utah

Crude protein digestibility. The percent digestibility of crude protein in sagebrush was highly variable (13.4% standard deviation) between trials. However, the mean DCP (54.0%) is very similar to earlier reports of 54.7% DCP by Utah workers (38, p. 26) and 52.4% DCP by Colorado investigators (51, p. 76). Smith (102, p. 288) found higher DCP (66.6%) from deer on pure sagebrush than in this study, but Bissell (18, p. 73) reported lower DCP (41.6%) from deer. The DCP (67.9%) for sagebrush when fed with the low protein mixed grass hay was greater than the other digestible protein percentages for sagebrush as determined by difference.

Ether extract digestibility. The ether extract of big sagebrush was more digestible (72.3%) than the ether extract found in either the alfalfa hays or the grass hay. This percentage ether extract digestibility for sagebrush is in agreement with several earlier reports (102, p. 288; 38, p. 26; 51, p. 76). Alfalfa and grass hays contain a lower percentage ether extract than sagebrush (Table 12), but the ether extract in alfalfa was not highly digestible (34.1% and 37.0%) whereas the ether extract in the grass hay was more digestible (65.9%), Table 14. The crude fat digestibility for alfalfa (green chop) was either negative or so minute that it was not included.

Crude fiber digestibility. The mean crude fiber digestibility (DCF) of 29.1% for sagebrush was considerably lower than either the DCF (39.5%) reported by Colorado workers (51, p. 76) or DCF (51.3%) reported by Smith (102, p. 288). The crude fiber of sagebrush was quite highly digestible (49.4%) when fed in combination with the alfalfa hay in Trial 1, but poorly digestible (12.0%) when fed with the grass hay in Trial 2. In Table 15, it is apparent that the crude fiber in sagebrush is more highly digestible when fed with substrates possessing poor crude fiber digestibilities than ones having higher digestibilities. Therefore, a possible interference with fiber digestibility could be present if the microorganisms are not supplied with an adequate supply of nutrients from other sources than sagebrush.

Cellulose digestibility. The mean digestibility of cellulose (52.2%) was higher than the mean crude fiber digestibility (29.1%); therefore, it appears that the rumen microorganisms are capable of decomposing the cellulose in sagebrush. In Trial 3, the combination of sagebrush and pelleted alfalfa hay even had a slightly higher digestibility (56.2%) than the complete alfalfa rations (55.0%) in Table 15. The mean cellulose digestibility (52.2%) reported here is greater than the DC (33.7%) reported by Cook and associates (105, p. 26).

Digestible energy. The mean digestible energy (53. 1%) for sagebrush was lower than the apparent digestibility coefficients of energy for the alfalfa hays and grass hays (Table 15). This DE (53. 1%) is also lower than the DE (70. 4%) which can be calculated from the data reported by Bissell et al. (18, p. 72). Since sagebrush is about 50% dry matter, large quantities of sagebrush must be ingested to meet an animal's energy requirement. Insufficient intake of energy due to low ingestion of sagebrush by deer could very easily result in starvation during severe winters.

Nitrogen-free extract digestibility. The mean digestibility of nitrogen-free extract (59. 2%) is less than the 78. 1% digestibility reported by Smith (102, p. 288), but higher than the 52. 1% nitrogen-free extract digestibility found in Colorado investigations (51, p. 76) and the 55. 9% reported by Utah workers (38, p. 26). The mean nitrogen-free extract digestibility for sagebrush (59. 2%) found in this study was lower than any of the digestibilities for the alfalfa and grass hays (Table 15). However, sagebrush contains 57. 2% nitrogen-free extract which makes it a good source of readily soluble carbohydrates (Table 12).

The DDM and DE of sagebrush were comparable and consistent in all digestion trials, and both indicate adequate utilization of sagebrush by sheep. The DCP in sagebrush was highly variable

and could be a limiting factor for body maintenance. Digestible ether extract was efficiently utilized by sheep and should serve as a good source of energy since sagebrush is relatively high in ether extract (7.0%). The digestibility of the nitrogen-free extract portion of sagebrush suggested that the sagebrush soluble carbohydrates are readily available to ruminants. The crude fiber digestibility was highly variable, and was also subnormal which may suggest a possible interference with microbial activity. Since the cellulose fraction was utilized much better, any detrimental effect must be selective.

Evaluation of Monthly Sagebrush Clippings

In addition to the previously discussed *in vivo* digestibility trials, which were not intended to measure seasonal variation, monthly sagebrush clippings were evaluated. The results from *in vitro* DDM, chemical analyses, and winter use of fertilized and unfertilized samples are presented here. The effects from adding essential oils to various substrates *in vitro* are also included.

In Vitro Dry Matter Digestibility

The *in vitro* DDM of monthly sagebrush clippings is given in Appendix, Table 7. These monthly data were then grouped into

seasonal digestibilities, Table 16, and are graphically presented in Figure 7. The in vitro DDM of sagebrush increased during the spring and then decreased in the late summer. The fertilized plots tended to have slightly greater in vitro DDM than the unfertilized, but clippings from the two collection sites also varied in their digestibility.

The predicted in vivo DDM, DE, and DCP percentages given in Table 16 were obtained from prediction equations for the in vitro DDM. The mean predicted DDM (56.0%), DE (54.8%), and DCP (51.7%) for the unfertilized sagebrush during the late summer were very similar to the actual in vivo DDM (54.5%), DE (53.1%), and DCP (54.0%) obtained in digestibility trials (Table 15). In Table 16, it is interesting to note that the summer period had the lowest predicted in vivo digestibilities. The predicted digestibilities of sagebrush were higher during the winter months when the predicted DDM and DE values approached those for alfalfa in Trials 1, 2, and 3 (Table 15).

Essential Oil Effects

In Table 17 the yields of steam volatile essential oils from sagebrush are given. The rate of fertilization had very little effect on the yield of essential oils; the mean for the unfertilized was 21.2

Table 16. Mean seasonal *in vitro* dry matter digestibility of sagebrush clippings, *in vivo* predictions therefrom, percent.

Seasons[6]	In Vitro		In Vivo					
	DDM		DDM[3]		DE[4]		DP[5]	
	U[1]	F[2]	U	F	U	F	U	F
Winter 1961-1962								
Plot 1	51.51	51.55	64.45	64.54	63.92	63.97	63.54	64.22
Plot 2	42.65	44.59	56.53	58.28	55.35	56.81	51.88	54.43
Late Spring 1962								
Plot 1	55.49	55.29	68.07	67.90	67.77	67.59	68.79	68.53
Plot 2	51.60	50.89	64.57	63.94	64.01	63.32	64.11	62.73
Late Summer 1962								
Plot 1	41.20	43.69	55.23	57.47	53.95	56.37	49.96	53.25
Plot 2	43.01	43.12	56.85	56.96	55.70	55.81	52.35	52.50
Winter 1962-1963								
Plot 1	46.17	47.31	59.70	60.72	58.76	59.86	56.52	58.01
Plot 2	47.91	51.06	61.26	64.09	60.44	63.49	58.80	62.61
Summary								
Plot 1 mean	48.60 ± 6.2	49.47 ± 5.1	61.87 ± 5.6	62.65 ± 4.5	61.13 ± 6.0	61.97 ± 4.9	59.70 ± 8.2	61.00 ± 6.7
Plot 2 mean	46.30 ± 4.3	47.42 ± 4.2	59.82 ± 3.8	60.82 ± 3.7	58.87 ± 4.1	59.85 ± 4.1	56.80 ± 5.8	58.05 ± 5.3
Pooled mean	47.45	48.45	60.85	61.74	60.00	60.91	58.25	59.53

1 Unfertilized

2 Fertilized with 16-20 ammonium phosphate.

3 $y = 18.2 + .8988\ X$

4 $y = 14.1 + .9673\ X$

5 $y = -4.3 + 1.3172\ X$

6 Plot 1, deep soil site; plot 2, hillside site.

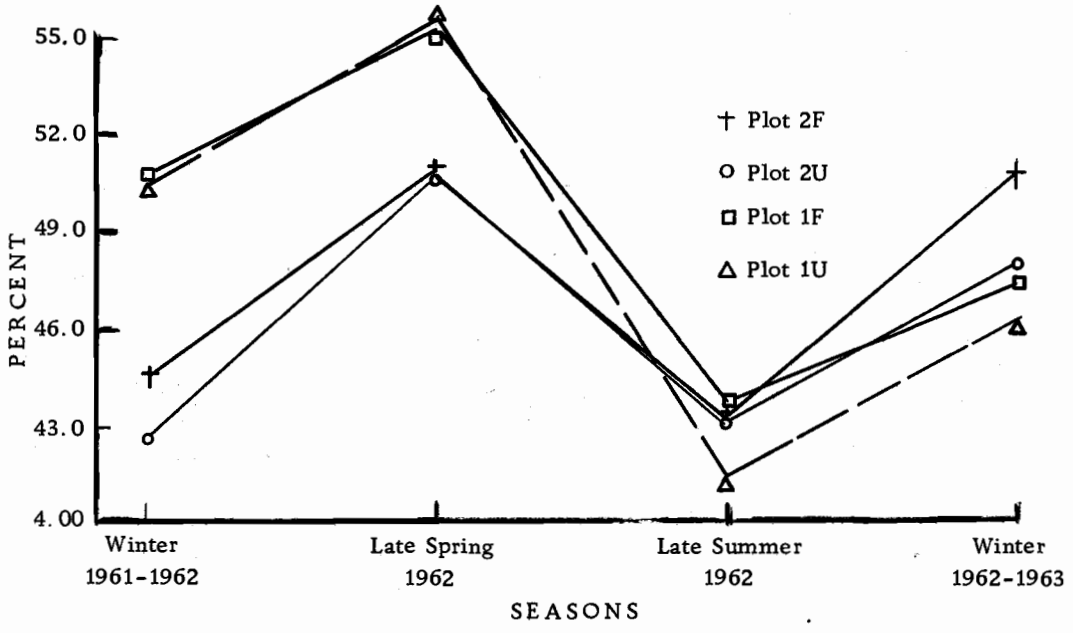

Figure 7. Seasonal trend in dry matter digestibility of current annual growth of sagebrush.

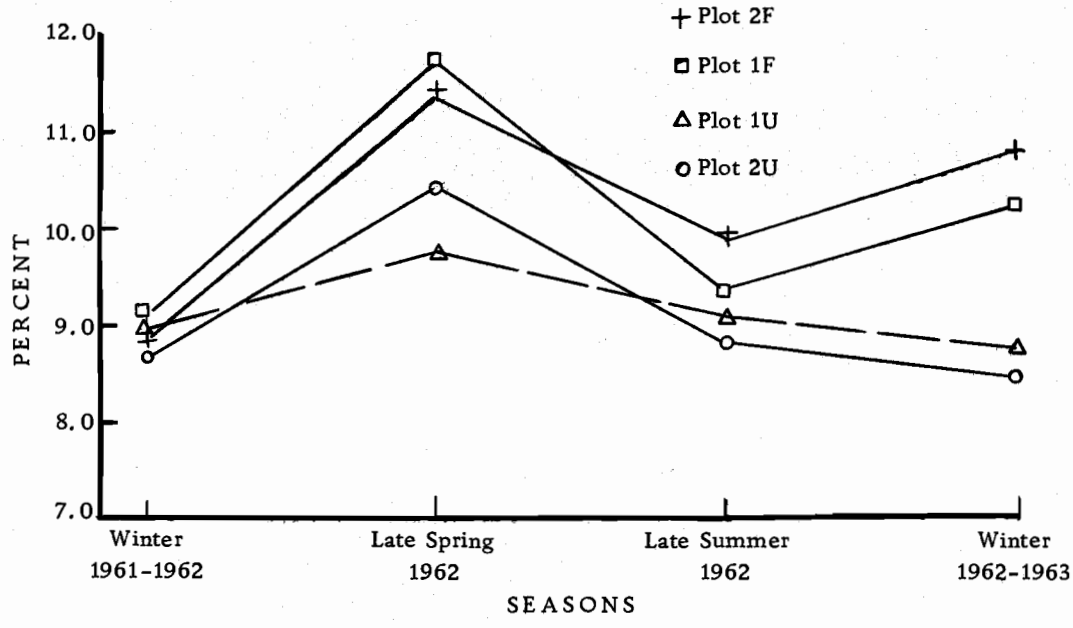

Figure 8. Seasonal trend in crude protein content of current annual growth of sagebrush, dry matter basis.

μl /gram and the mean for the fertilized was 21.9 μl /gram (Table 17).

It was suggested in the literature review that essential oils may inhibit microbial activity (18, p. 57-78). The addition of essential oils to various substrates was undertaken to study these inhibitory effects. The in vitro DDM responses from the addition of 30, 50, and 100 μl of essential oils are given for one gram samples of each substrate (Table 18). The 30 μl level of oil is about that ordinarily found in sagebrush (Table 17); the poorer quality grass hays were the only substrates that had decreased digestion with the 30 μl level of essential oils. The addition of 50 μl slightly decreased the digestion of the two alfalfa samples and significantly decreased the digestibility of the poorer quality grass hays. The addition of 100 μl of oil, three times that normally found in sagebrush, decreased in vitro DDM in the alfalfa and grass hays, but quite satisfactory in vitro DDM was still present; so many of the microorganisms must have been unaffected.

A selective effect from the essential oils is suggested because in vitro DDM of sagebrush (which already contains essential oils) was not adversely affected by even the 100 μl level, whereas all other substrates were affected with the 50 μl level. Essential oils are known to possess specific rather than general

inhibitory effects on microorganisms (60, p. 77-83). Therefore, the oils may be impairing organisms that are not required in the fermentation of sagebrush.

Table 17. Yield of essential oils from sagebrush clippings.[1]

Month	µl gram dry matter[2]	
	unfertilized	fertilized
April 1962	15.4	18.3
June 1962	25.4	29.6
August 1962	26.3	33.1
October 1962	24.4	23.1
December 1962	25.4	22.3
January 1963	23.7	22.5
February 1963	17.4	13.6
March 1963	11.2	12.5
Mean	21.2±5.7	21.9±7.1

1. Clippings were taken from a Forested Site.
2. Yield expressed as microliters essential oil per gram sagebrush.

Chemical Composition

Monthly analyses of sagebrush clippings from both fertilized and unfertilized plots are given in the Appendix, Table 6. These monthly clippings were grouped by seasons, and the seasonal chemical analyses are given in Table 19. The seasons were grouped as follows: winter 1961-1962 included November and December 1961 and January and February 1962; late spring 1962 included March, April and May; late summer 1962 included June, July, August and

Table 18. Effect of added essential oils in vitro dry matter digestion, percent.

SUBSTRATE	Sagebrush essential oils			
	0	30 µl	50 µl	100 µl
Alfalfa Hay				
Mean %[1]	47.15	48.13	46.43	41.25
S. D. %[2]	1.45	.50	.78	1.96
C. V. %	3.08	1.04	1.68	4.75
Alfalfa (green chop)				
Mean %	47.68	47.70	46.00	41.83
S. D. %	1.33	.13	2.22	.87
C. V. %	2.78	.27	4.83	2.08
Meadow Grass Hay 1				
Mean%	46.98	45.00	39.23	30.75
S. D. %	.61	.24	.43	.39
C. V. %	1.30	.53	1.10	1.27
Meadow Grass Hay 2				
Mean %	41.40	37.80	27.95	26.35
S. D. %	1.23	.79	3.42	.24
C. V. %	2.97	2.35	12.24	.91
Meadow Grass Hay 3				
Mean %	35.63	32.28	26.03	24.55
S. D. %	.62	1.27	.94	.44
C. V. %	1.74	3.93	3.61	1.79
Meadow Grass Hay 4				
Mean %	33.28	30.23	23.90	22.43
S. D. %	1.10	.50	.29	.33
C. V. %	3.31	1.65	1.21	1.47
Mixed Grass Hay(OSU)				
Mean %	42.80	38.20	37.58	32.78
S. D. %	1.01	.50	.35	.70
C. V. %	2.36	1.31	.93	2.14
Sagebrush				
Mean %	40.00	39.60	39.73	39.88
S. D. %	.47	.52	.99	.17
C. V. %	1.18	1.31	2.49	.43

[1] Standard deviation

[2] Coefficient of variation

Table 19. Chemical analyses of sagebrush monthly clippings of sagebrush. collected near Silver Lake, Oregon.[1]

Seasons[4]	Crude Protein		Crude Fiber		Ether Extract		Ash		Energy	
	U[2]	F[3]	U	F	U	F	U	F	U	F
Winter 1961-1962										
Plot 1	8.99	9.08	18.49	18.09	16.80	17.94	2.87	3.09	5.34	5.39
Plot 2	8.68	8.91	19.69	20.24	14.44	14.31	2.94	3.03	5.66	5.82
Late Spring 1962										
Plot 1	9.76	11.78	16.02	16.55	13.81	14.10	3.17	3.22	5.46	5.49
Plot 2	10.44	11.37	17.07	16.85	13.48	12.74	3.24	3.61	5.43	5.43
Late Summer 1962										
Plot 1	9.06	9.38	22.37	21.95	11.17	12.71	3.81	4.11	5.14	5.31
Plot 2	8.80	9.93	22.50	22.02	13.24	14.07	3.85	4.37	5.33	5.57
Winter 1962-1963										
Plot 1	8.73	10.25	19.11	19.16	14.41	14.29	3.24	2.94	5.43	5.72
Plot 2	8.49	10.80	19.64	18.88	14.07	14.66	3.11	3.08	5.60	5.56
Summary										
Plot 1 mean	9.14 ± 0.4	10.12 ± 1.2	19.00 ± 2.6	18.98 ± 2.3	14.05 ± 2.3	14.75 ± 1.9	3.27 ± 0.4	3.34 ± 0.5	5.34 ± 0.1	5.48 ± 0.2
Plot 2 mean	9.10 ± 0.9	10.06 ± 1.0	19.72 ± 2.2	19.50 ± 2.1	13.80 ± 0.5	13.93 ± 0.9	3.28 ± 0.4	3.52 ± 0.6	5.51 ± 0.1	5.60 ± 0.2
Pooled mean	9.12	10.09	19.36	19.24	13.93	14.34	3.28	3.43	5.43	5.54

1 Percents are on a dry matter basis.
2 Unfertilized.
3 Fertilized with 16-20 ammonium phosphate
4 Plot 1, deep soil site; plot 2, hillside plot.

Table 19 (Continued)

Seasons	NFE		Moisture		Cellulose		Calcium		Phosphorus	
	U	F	U	F	U	F	U	F	U	F
Winter 1961-1962										
Plot 1	53.70	51.81	50.6	54.0	21.2	21.0	.32	.28	.24	.25
Plot 2	54.24	53.50	53.6	54.1	16.9	18.0	.38	.36	.23	.23
Late Spring 1962										
Plot 1	56.88	54.35	47.8	50.4	17.5	17.8	.39	.36	.22	.22
Plot 2	55.45	55.42	43.2	43.0	16.7	17.3	.45	.45	.24	.25
Late Summer 1962										
Plot 1	53.60	51.85	52.3	52.2	20.3	18.7	.39	.35	.28	.31
Plot 2	51.60	49.61	48.8	50.3	19.7	19.1	.38	.37	.27	.27
Winter 1962-1963										
Plot 1	54.51	53.35	51.6	51.7	17.1	16.9	.44	.36	.23	.24
Plot 2	54.69	53.02	48.4	45.1	17.4	16.6	.37	.42	.22	.24
Summary										
Plot 1 mean	54.67 ± 1.5	52.87 ± 1.3	50.6 ± 2.0	52.1 ± 1.5	19.0 ± 2.0	18.7 ± 1.7	.38 ± 0.1	.34 ± 0.1	.24 ± 0.1	.25 ± 0.1
Plot 2 mean	54.00 ± 1.7	52.87 ± 2.4	48.5 ± 3.7	48.1 ± 5.0	17.7 ± 1.4	17.6 ± 1.1	.40 ± 0.1	.40 ± 0.1	.24 ± 0.1	.25 ± 0.1
Pooled mean	54.33	52.87	49.6	50.1	18.4	18.2	.39	.37	.24	.25

October; and winter 1962-1963 included December 1962, January, February and March 1963. No samples were collected in either September or November 1962.

Crude protein. The seasonal variation in crude protein is graphically presented in Figure 8. Percent crude protein tended to decrease during the dormant winter period, and then increased with the growth of sagebrush in the spring. The low protein period during the winter probably is not too critical since sagebrush had above 8.5% crude protein in both of the winters covered (Table 19). As would be expected, fertilization with a nitrate fertilizer did increase the crude protein content of the sagebrush, from approximately 9% for the unfertilized to 10% for the fertilized sagebrush during the winter of 1962-1963.

Gross energy and ether extract. The percent ether extract and the kilocalories of gross energy in sagebrush followed a similar trend (Figures 9 and 10). Due to the variation in both ether extract and energy it is difficult to pinpoint the effect of fertilization. Mean values of 5.43 kilocalories per gram for the unfertilized and 5.54 kilocalories for the fertilized plots (Table 19) suggest a possible increase in energy, but the fertilization probably had its effect rather in increasing yield than in changing the gross energy (KCal/gm). It should also be noted that sagebrush is high in energy and

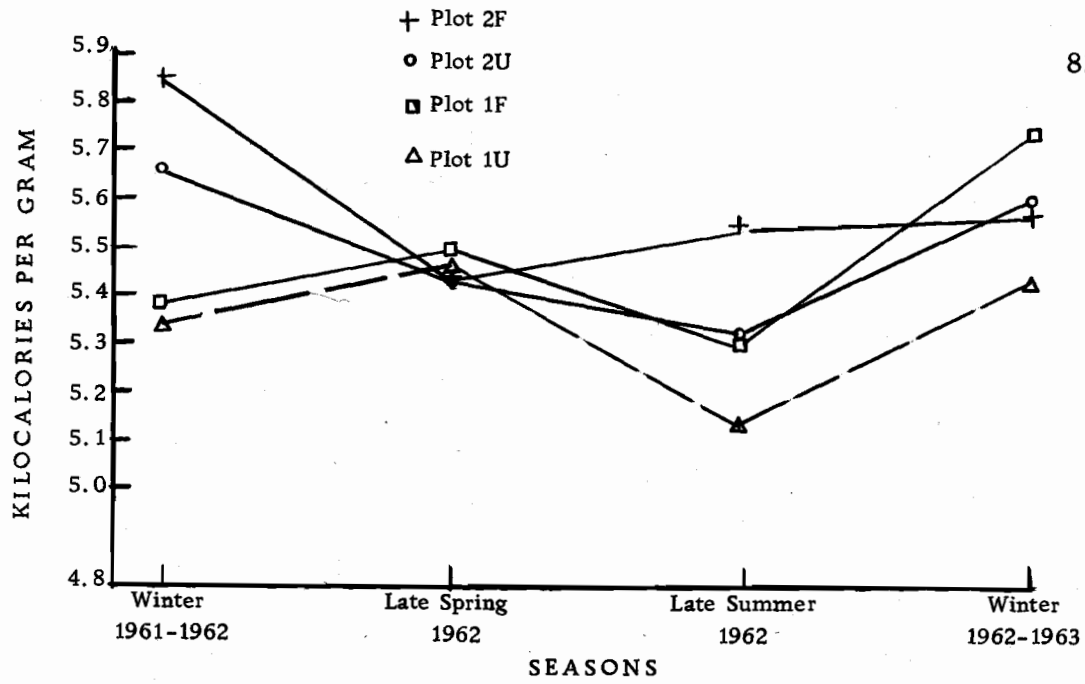

Figure 9. Seasonal trend in gross energy content of current annual growth of sagebrush, dry matter basis.

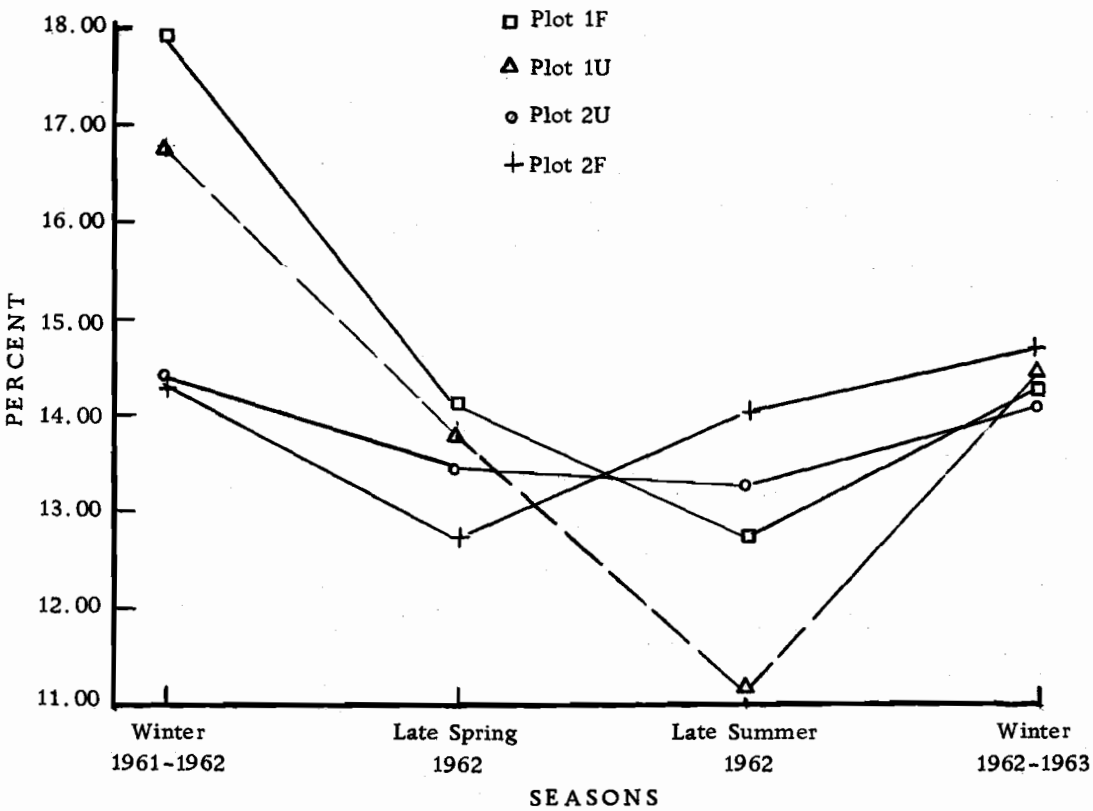

Figure 10. Seasonal trend in ether extract content of current annual growth of sagebrush, dry matter basis.

ether extract, which if properly utilized, could be invaluable to deer during severe winters.

Crude fiber and cellulose. The percent crude fiber and cellulose followed similar trends (Figures 11 and 12). Percent crude fiber and cellulose were low in the spring, increased in the late summer, and then decreased in the winter. The twigs, analyzed in this study (Table 19), were not high in either crude fiber (maximum 22.5%) or cellulose (maximum 21.2%). Fertilization did not markedly affect the percent crude fiber or cellulose: if any at all, the effect was a slight decrease in both.

Nitrogen-free extract. Nitrogen-free extract (NFE) percentages are given in Table 19 for the monthly sagebrush clippings and are graphically presented in Figure 12. The data suggested a trend toward decreased NFE as a result of fertilization which is probably a function of increased crude protein and ether extract in the fertilized samples. The NFE of plants represents the soluble carbohydrates which are readily available to animals. The values presented here are similar to those reported by Colorado investigators (50, p. 121); the NFE of sagebrush was below that for other browse species (50, p. 121).

Ash, calcium and phosphorus. The percent ash in sagebrush is given in Table 19 and presented graphically in Figure 14.

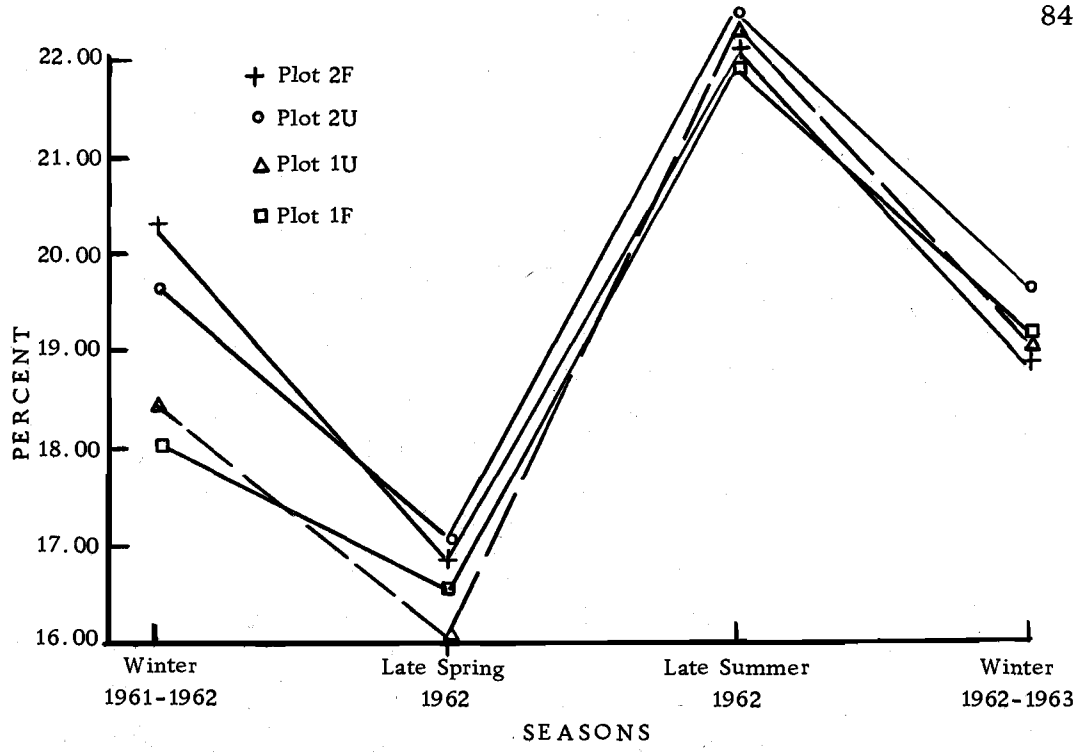

Figure 11. Seasonal trend on the crude fiber content of current annual growth of sagebrush, dry matter basis.

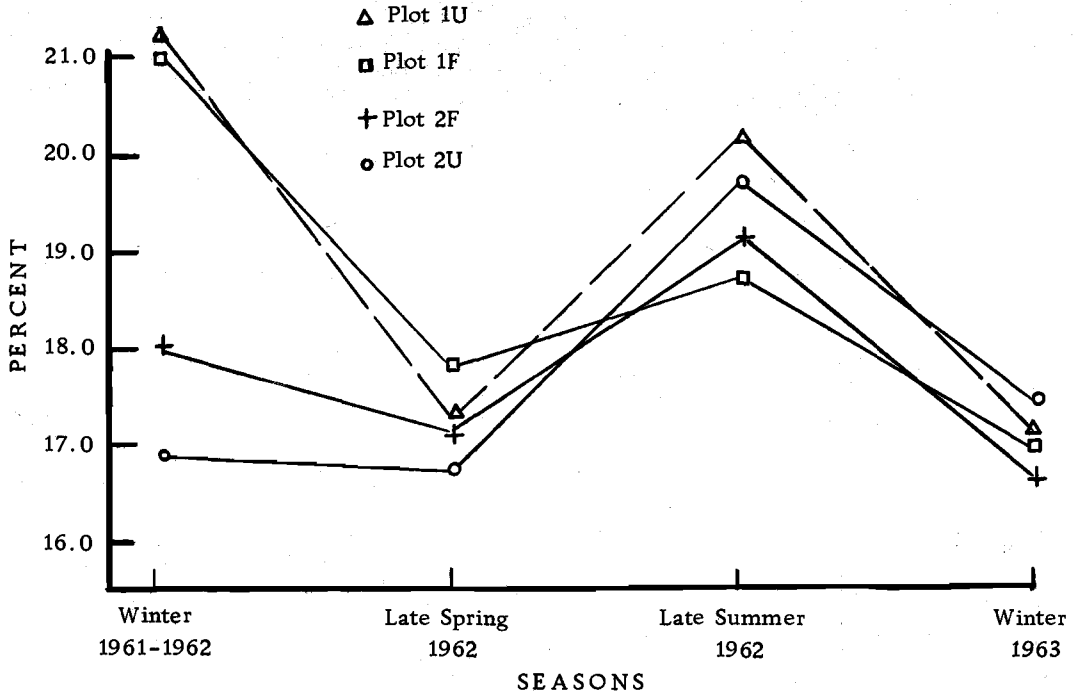

Figure 12. Seasonal trend in the cellulose content of current annual growth of sagebrush, dry matter basis.

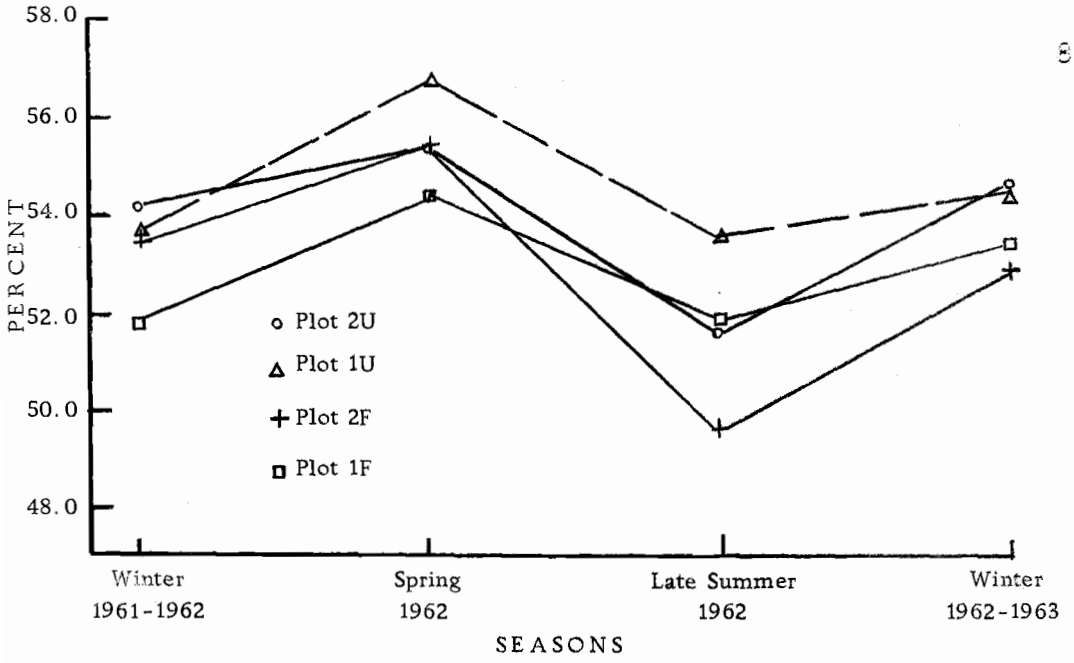

Figure 13. Seasonal trend in the nitrogen-free extract content of the current annual growth of sagebrush, dry matter basis.

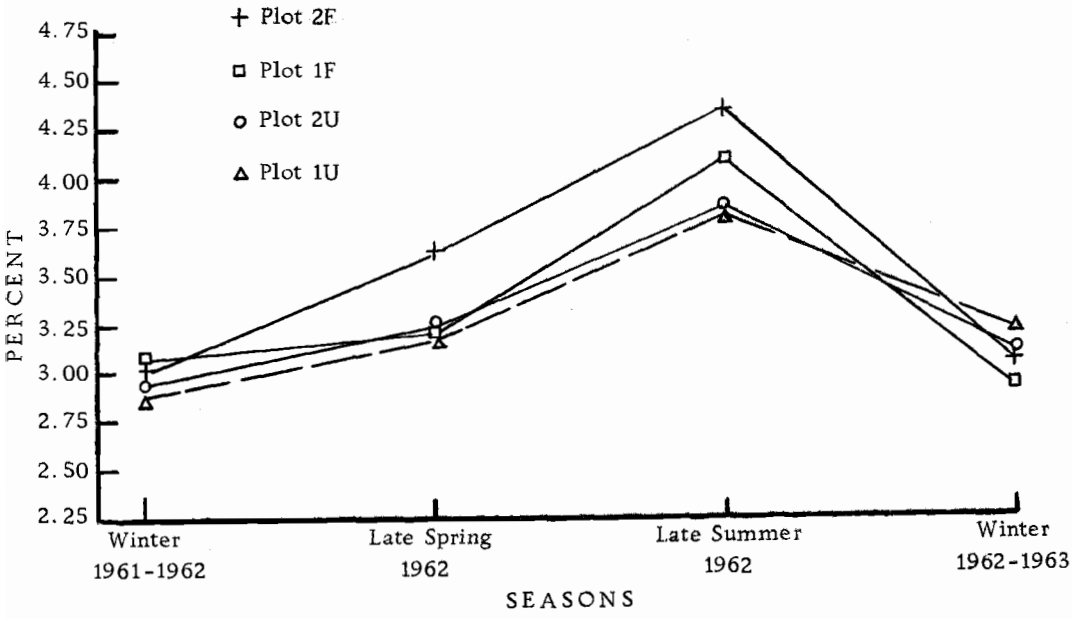

Figure 14. Seasonal trend in the ash content of the current annual growth of sagebrush, dry matter basis.

Percent ash followed the same trend in both the fertilized and unfertilized plots. Ash apparently reached a peak in the late fall, and decreased during the winter and spring.

The values for calcium and phosphorus are presented in Figures 15 and 16). The calcium content increased in the spring, and then decreased to a level that was maintained during the summer and winter. Colorado workers (50, p. 119-120) reported mean values for both calcium (0.81%) and phosphorus (0.32%) which are higher than those obtained in this study for either calcium (0.38%) or phosphorus (0.25%). An increase in the calcium and phosphorus content resulting from fertilization with a nitrate-phosphate fertilizer was not apparent in this study.

Winter Use of Sagebrush

The data in Table 20 represent the intensity of winter use by deer of tagged sagebrush twigs on fertilized and unfertilized sites. The use during the first winter (1961-1962) was not dependent on the rate of fertilization since it was applied too late in the year to affect growth. However, the fertilized plots tended to be used more than the unfertilized ones during the second winter (1962-1963), which was a winter of light deer use (Table 20). Possible explanations for the greater use of the fertilized sagebrush are as follows:

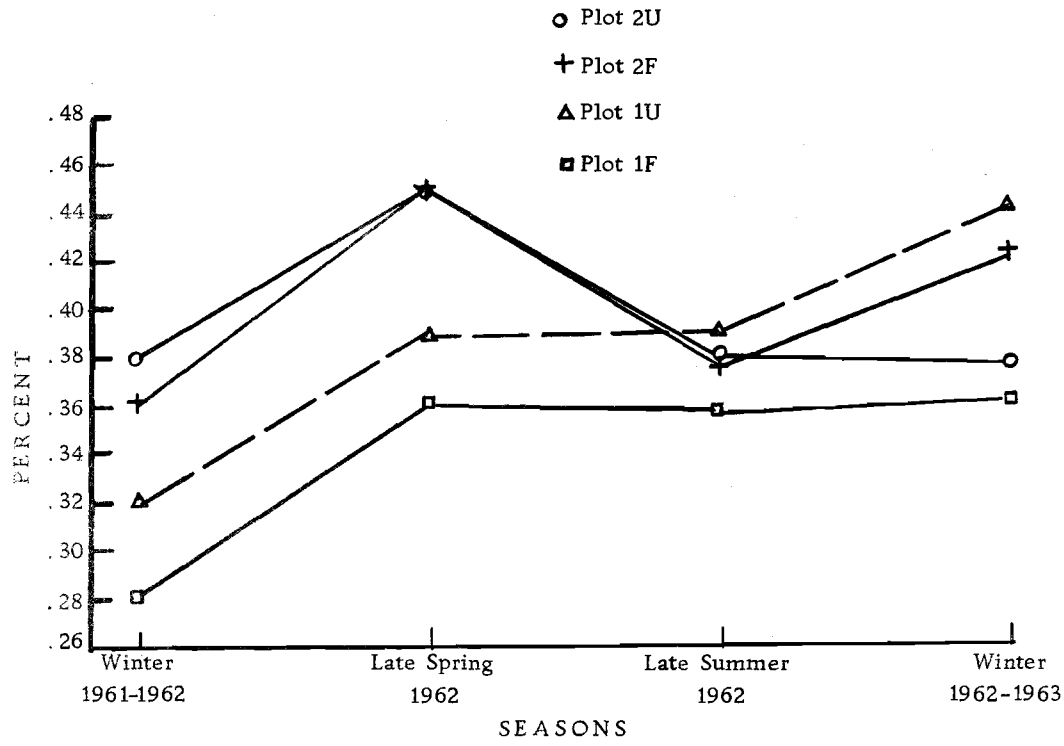

Figure 15. Seasonal trend in the calcium content of the current annual growth of sagebrush, dry matter basis.

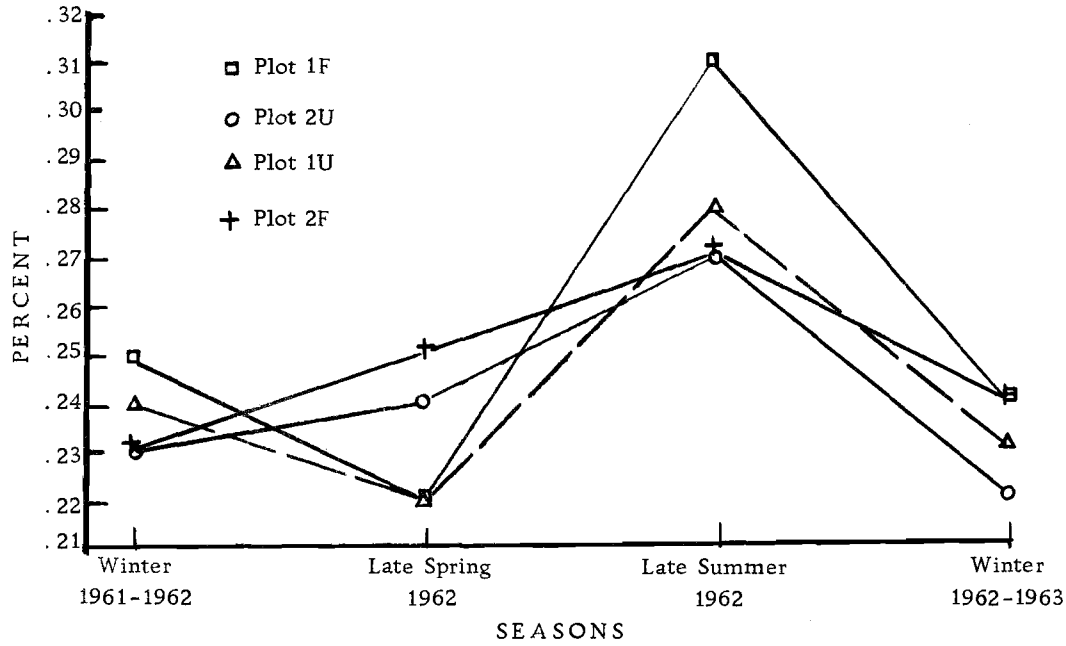

Figure 16. Seasonal trend in the phosphorus content of the current annual growth of sagebrush, dry matter basis.

Table 20. Winter use of sagebrush at Silver Lake, Oregon, determined from twig length measurements.

PLOT	Average twig length, Fall (inches)	Average twig length, Spring (inches)	Intensity of Use (Percent)
Winter 1961-1962[A]			
Unfertilized			
Plot 1	2.13	1.88	11.74
Plot 2	1.47	1.22	17.01
Plot 3	2.36	1.81	23.31
Mean			17.35
Fertilized			
Plot 1	1.96	1.79	8.67
Plot 2	1.47	1.22	17.01
Plot 3	2.36	1.81	23.31
Mean			16.33
Winter 1962-1963[B]			
Unfertilized			
Plot 1	1.79	1.78	.56
Plot 2	1.23	1.21	1.63
Plot 3	1.93	1.46	24.35
Mean			8.85
Fertilized			
Plot 1	2.31	2.24	3.03
Plot 2	2.01	1.81	9.95
Plot 3	2.26	1.29	42.92
Mean			18.63

[A] Each value represents an average of 25 twigs.

[B] Each value represents an average of 25 twigs with the following exceptions: (1) plot 1 unfertilized represents 24 twigs (2) plot 3 unfertilized represents 22 twigs, and (3) plot 3 fertilized represents only 14 twigs.

(1) fertilized sagebrush twigs were longer than the unfertilized due to a greater growth of the fertilized sage (Table 20), (2) fertilized sagebrush was markedly higher in crude protein and slightly higher in gross energy (Table 19) and (3) the fertilized sagebrush tended to be higher in digestibility as determined in an artificial rumen.

One might assume that lowering the essential oil content would increase the digestibility and use of sagebrush, but the data in Table 17 do not support this hypothesis; the fertilized and unfertilized samples are similar in essential oil content. The intensity of use values given in this section for deer use are similar to a 16% mean use value for sheep use reported by Cook and associates (38, p. 26).

SUMMARY

1. Two in vivo digestion trials were conducted using alfalfa and a mixed grass hay fed singly and in combination with various percentages of sagebrush. A third digestibility trial included pelleted alfalfa hay fed singly and in combination with sagebrush which was forced into the rumens via fistulas. Digestion coefficients were obtained for sagebrush (by difference) from ten lambs.

2. Consumption of sagebrush by lambs weighing 75-100 pounds varied from 75 to 306 grams per day compared to 500 to 900 grams per day for the other forages. The body weight changes were not adversely affected by the amounts of sagebrush fed in these trials.

3. Mean digestibility coefficients for dry matter (54.5%) and energy (53.1%) of sagebrush were comparable and consistent in all digestion trials, and both indicate adequate utilization of sagebrush by sheep. On the other hand, digestibility coefficients for protein in sagebrush were highly variable (13.4% standard deviation), and could suggest a limiting factor in body maintenance. The ether extract was highly digested (72.3%) by sheep; it should be a good source of energy since this sagebrush contained seven percent ether extract. The mean digestibility of the nitrogen-free extract portion (59.2%) of sagebrush suggests that the soluble carbohydrates

are readily available to ruminants. Whereas, the mean crude fiber digestibility (29. 1%) was highly variable and suboptimal.

4. An artificial rumen was used to study the effects of essential oils on microbial multiplication. A selective effect from these oils is suggested since in vitro dry matter digestibility for sagebrush (which already contains essential oils) was not adversely affected by even the 100 µl levels of oil supplementation, whereas, all other substrates were affected by the 50 µl level. The yield of essential oils was determined for 18 samples of sagebrush, and ranged from 11. 2 to 33 µl/gram.

5. Several factors were employed to evaluate the versatility of the artificial rumen technique used in this study. The mean, standard deviation and coefficient of variation were calculated for each of the 384 treatment combinations of substrate, rumen fluid, position of flask, and source of inoculum. Results from the analysis of variance indicate that forages of varying nutritional value may be differentiated by in vitro dry matter digestibility. Furthermore, a procedure which is repeatable has been used with inoculum from both sheep and cattle. Increasing the level of rumen liquor from 5 to 15 ml resulted in greater dry matter digestibility in vitro, but to a greater degree with inoculum from cattle than from sheep.

6. Significant correlation coefficients from nine substrates were

obtained between *in vitro* dry matter digestibility and *in vivo* digestibilities of energy, dry matter, organic matter, and protein; simple regression equations are given for each of these entities as obtained with 5 and 15 ml of rumen fluid from both sheep and cattle. The *in vitro* and predicted *in vivo* values, obtained from microbial fermentations of monthly sagebrush twigs, suggest that they should be quite highly digestible by ruminants.

7. Three fertilized and unfertilized plots of sagebrush were established near Silver Lake, Oregon. Monthly analyses of clippings from these plots showed that nitrogen and phosphate fertilization resulted in an increase in crude protein, *in vitro* dry matter digestibility, and growth; whereas, the percent nitrogen-free extract was decreased. Apparently, fertilization did not affect the percentages of cellulose, crude fiber, ash, calcium, and phosphorus. The intensity of winter use of tagged sagebrush twigs by deer was greater on fertilized than unfertilized plots.

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APPENDIX

Appendix. Table 1. Mean in vitro dry matter digestibilities for rumen fluid level times source of inoculum interaction.

Inoculum Source	Rumen Liquor		Inoculum Mean
	5 ml	15 ml	
Cattle	40.94	46.94	43.94
Sheep	42.90	43.38	43.14
Rumen liquor mean	41.92	45.16	

Appendix, Table 2. Mean in vitro dry matter digestibilities for rumen fluid times substrate interaction.

SUBSTRATE	Rumen Liquor		SUBSTRATE MEANS
	5 ml	15 ml	
Alfalfa Hay	45.59	49.97	47.78
Alfalfa (green chop)	52.05	49.97	51.01
Meadow Grass Hay 1	47.70	52.18	49.94
Meadow Grass Hay 2	36.40	40.40	42.94
Meadow Grass Hay 3	40.97	44.90	38.22
Meadow Grass Hay 4	34.38	38.75	36.56
Mixed Grass Hay	43.98	49.26	46.62
Sagebrush	34.33	36.20	35.26
Rumen liquor means	41.92	45.16	

Appendix. Table 3. Mean in vitro dry matter digestibilities for source of inoculum times substrate interaction.

SUBSTRATE	Source of Inoculum	
	Cattle	Sheep
Alfalfa Hay	46.68	48.88
Alfalfa (green chop)	51.78	50.24
Meadow Grass Hay 1	52.83	47.05
Meadow Grass Hay 2	44.53	41.34
Meadow Grass Hay 3	38.63	37.82
Meadow Grass Hay 4	36.90	36.22
Mixed Grass Hay	46.95	46.29
Sagebrush	33.22	37.30

Appendix, Table 4. Mean in vitro dry matter digestibilities for rumen fluid times location of flask interaction.

Location of flasks	Rumen liquor		Location means
	5 ml	15 ml	
Position 1	41.91	44.18	43.05
Position 2	42.14	45.35	43.75
Position 3	42.04	45.31	43.68
Position 4	41.60	45.79	43.69

Appendix, Table 5. Mean in vitro dry matter digestibilities for location of flask times substrate interaction.

SUBSTRATE	Location of flasks			
	Position 1	Position 2	Position 3	Position 4
Alfalfa Hay	47.80	47.34	48.28	47.69
Alfalfa (green chop)	50.93	51.21	50.33	51.58
Meadow Grass Hay 1	48.90	50.72	49.99	50.17
Meadow Gass Hay 2	42.30	42.98	43.25	43.21
Meadow Grass Hay 3	37.58	38.38	38.70	38.23
Meadow Grass Hay 4	35.91	37.24	36.58	36.52
Mixed Grass Hay	45.87	46.74	46.84	47.03
Sagebrush	35.10	35.38	35.43	35.13

Appendix, Table 6. Chemical Analyses of Monthly Sagebrush Clippings at Silver Lake, Oregon, Percent.[1]

		Crude Protein		Crude Fiber		Crude Fat		Cellulose		Gross Energy		NFE		Ash		Calcium		Phosphorus		Total Dry Matter	
Date	Plot[1]	U[2]	F[3]	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F
Nov. 1961	1	8.26	8.19	18.30	17.69	16.42	17.17	21.19	21.55	5.14	5.38	53.53	53.61	3.49	3.34	.313	.295	.261	.221	59.2	58.6
	2	8.59	9.03	20.51	18.88	13.00	13.54	16.96	16.85	5.65	5.66	54.64	55.30	3.26	3.25	.378	.328	.244	.237	58.3	56.7
Dec. 1961	1	9.38	9.72	20.06	18.76	17.72	16.68	23.71	23.08	5.31	5.31	53.50	51.54	2.73	3.30	.358	.333	.224	.198	48.7	52.4
	2	8.95	9.25	19.96	22.75	14.76	14.91	17.14	20.27	5.73	6.28	53.38	49.99	3.04	3.10	.333	.369	.224	.240	50.6	50.6
Jan. 1962	1	9.21	9.50	17.72	17.05	16.97	17.72	20.88	20.03	5.27	5.25	53.26	52.34	2.84	3.39	.313	.292	.167	.198	43.1	53.2
	2	8.50	8.46	18.61	19.09	15.66	14.49	16.62	17.01	5.59	5.53	54.71	55.21	2.52	2.75	.426	.374	.211	.217	51.8	55.1
Feb. 1962	1	9.10	8.90	17.88	18.85	16.10	20.20	18.91	19.40	5.65	5.63	54.50	49.73	2.42	2.32	.302	.190	.304	.203	51.3	51.9
	2																				
Mar. 1962	1	8.88	9.22	17.14	17.96	17.91	16.98	18.84	18.79	5.70	5.73	53.50	53.48	2.57	2.36	.309	.287	.209	.200	51.1	51.1
	2	8.75	8.85	19.41	19.10	16.21	13.60	17.55	17.42	5.62	5.52	53.23	55.55	2.40	2.90	.378	.396	.188	.206	53.7	52.9
Apr. 1962	1	9.87	11.43	17.04	15.98	13.39	14.87	19.21	19.06	5.51	5.64	56.63	54.28	3.07	3.44	.373	.326	.186	.189	51.1	61.5
	2	11.04	12.13	16.13	17.06	16.09	14.47	16.98	18.06	5.58	5.57	53.06	52.51	3.68	3.83	.431	.418	.249	.261	41.8	41.2
May 1962	1	10.52	14.70	13.88	15.71	11.22	10.46	14.48	15.53	5.17	5.10	60.50	55.28	3.88	3.85	.487	.467	.266	.279	41.1	38.5
	2	11.52	13.14	15.67	14.38	9.13	10.16	15.60	16.51	5.14	5.21	60.05	58.21	3.63	4.11	.589	.539	.280	.280	34.2	34.9
June 1962	1	10.29	9.37	23.54	22.21	8.18	10.41	21.71	18.69	5.03	5.04	53.68	53.19	4.31	4.82	.371	.340	.341	.393	52.0	46.0
	2																				
July 1962	1	8.40	9.76	23.76	21.43	11.10	12.90	21.28	20.36	5.11	5.13	52.61	51.45	4.13	4.46	.397	.349	.273	.291	49.2	47.5
	2	10.24	11.03	24.25	24.19	11.26	15.24	21.07	20.02	5.21	5.45	49.67	43.79	4.58	5.75	.346	.348	.304	.309	38.8	43.8
Aug. 1962	1	8.05	8.75	24.40	24.08	13.42	14.56	21.46	20.02	5.41	5.30	50.11	48.48	4.02	4.13	.385	.361	.349	.326	59.2	61.8
	2	7.67	8.89	22.66	21.20	13.46	15.13	20.06	19.66	5.28	5.52	52.72	51.15	3.49	3.63	.422	.394	.274	.255	56.3	53.6
Oct. 1962	1	9.50	9.64	17.76	20.06	11.97	12.98	16.59	15.77	5.01	5.78	58.01	54.28	2.76	3.04	.392	.336	.226	.212	48.9	53.5
	2	8.50	9.86	20.60	20.67	14.99	11.85	17.85	17.59	5.49	5.75	52.42	53.90	3.49	3.72	.380	.372	.216	.241	51.4	53.5
Dec. 1962	1	8.51	9.96	19.31	21.01	14.60	12.34	18.23	18.02	5.21	5.80	54.21	53.83	3.37	2.86	.416	.426	.240	.222	54.2	53.1
	2	7.35	9.06	20.56	20.37	12.41	15.82	17.22	16.17	5.51	5.59	56.76	51.61	2.92	3.14	.342	.384	.206	.241	37.9	35.9
Jan. 1963	1	8.66	9.39	20.10	19.44	14.06	13.97	16.42	16.92	5.25	5.68	53.01	53.70	4.17	3.50	.339	.304	.262	.269	55.1	60.0
	2	8.31	9.45	19.68	18.75	15.47	14.03	17.72	16.75	5.44	5.51	53.48	54.83	3.06	2.94	.340	.360	.191	.211	58.0	54.0
Feb. 1963	1	8.31	10.75	18.76	17.18	15.05	15.46	16.58	15.79	5.52	5.62	55.45	54.05	2.43	2.56	.502	.311	.205	.227	46.2	43.3
	2	8.86	11.45	19.16	17.21	16.12	14.42	16.93	15.64	5.68	5.53	52.82	53.76	3.04	3.16	.389	.472	.247	.255	45.4	40.7
Mar. 1963	1	9.45	10.91	18.25	19.02	13.93	15.40	17.12	16.73	5.72	5.78	55.37	51.83	3.00	2.84	.483	.412	.224	.221	50.9	50.3
	2	9.43	11.49	19.16	19.17	12.27	14.38	17.55	17.50	5.76	5.61	55.71	51.88	3.43	3.08	.408	.473	.227	.236	52.2	49.8

1. Percent is on Dry Matter Basis
2. Unfertilized
3. Fertilized with 16-20 ammonium phosphate
4. Plot 1. deep soil site; plot 2. hillside site

Appendix, Table 7. In vitro dry matter digestibility of monthly sagebrush clippings, in vivo predictions therefrom, percent.

Date	Plot[6]	In vitro DDM U[1]	In vitro DDM F[2]	Predicted DDM[3] U	Predicted DDM[3] F	Predicted DE[4] U	Predicted DE[4] F	Predicted DD[5] U	Predicted DD[5] F
Nov. 1961	1	52.78 ± .19	52.30 ± .47	65.46	65.21	65.15	64.69	65.22	64.59
	2	40.12 ± .43	51.55 ± 1.05	54.26	64.53	52.91	63.69	48.55	63.60
Dec. 1961	1	48.23 ± .40	52.33 ± .29	61.55	65.23	60.75	64.71	59.23	64.63
	2	41.00 ± .81	34.70 ± 1.30	55.05	49.39	53.76	46.67	49.71	41.41
Jan. 1962	1	52.58 ± .26	51.43 ± 1.05	65.46	64.43	64.96	63.85	64.96	63.44
	2	46.82 ± .35	47.52 ± 1.04	60.28	60.91	59.39	60.07	57.37	58.29
Feb. 1962	1	52.43 ± .88	50.15 ± .64	65.32	63.27	64.82	62.61	64.76	61.72
	2								
Mar. 1962	1	52.88 ± .50	55.15 ± .24	65.73	67.77	65.25	67.45	65.35	68.34
	2	46.22 ± .38	49.72 ± .49	59.74	62.89	58.81	62.19	56.58	61.19
Apr. 1962	1	54.45 ± .33	52.18 ± .38	67.14	65.10	66.77	64.57	67.42	64.43
	2	50.22 ± .24	47.82 ± .10	63.34	61.18	62.68	60.36	61.85	58.69
May 1962	1	59.13 ± .41	58.55 ± .21	71.35	70.82	71.30	70.74	73.59	72.82
	2	58.35 ± .35	55.12 ± .10	70.64	67.74	70.54	67.42	72.56	68.30
June 1962	1	46.33 ± .38	49.05 ± .24	59.84	62.29	58.92	61.55	56.73	60.31
	2								
July 1962	1	40.40 ± .75	41.08 ± .46	54.51	55.12	53.18	53.84	48.91	49.81
	2	43.30 ± .54	42.72 ± .17	57.12	56.60	55.98	55.42	52.73	51.97
Aug. 1962	1	38.00 ± .64	42.92 ± .19	52.35	56.78	50.86	55.62	45.75	52.23
	2	39.70 ± .38	42.00 ± .43	53.88	55.95	52.50	54.73	47.99	51.02
Oct. 1962	1	40.05 ± .45	41.72 ± .51	54.20	55.70	52.84	54.46	48.45	50.65
	2	46.02 ± .50	44.65 ± .41	59.56	58.33	58.62	57.29	56.32	54.51
Dec. 1962	1	43.02 ± .51	43.80 ± .20	56.87	57.57	55.71	56.47	52.37	53.39
	2	44.75 ± .31	47.40 ± .14	58.42	60.80	57.39	59.95	54.64	58.14
Jan. 1963	1	41.52 ± .76	45.60 ± .14	55.52	59.19	54.26	58.21	50.39	55.76
	2	49.07 ± .84	50.15 ± .67	62.30	63.27	61.57	62.61	60.34	61.76
Feb. 1963	1	50.82 ± .38	50.00 ± .24	63.88	63.14	63.26	62.47	62.64	61.56
	2	47.80 ± .32	55.87 ± .38	61.16	68.42	60.34	68.14	58.66	69.29
Mar. 1963	1	49.32 ± .52	49.82 ± .33	62.53	62.97	61.81	62.29	60.66	61.32
	2	50.00 ± 1.00	50.80 ± .73	63.14	63.86	62.47	63.24	61.56	62.61

1. Unfertilized
2. Fertilized with 16-20 ammonium phosphate
3. $y = 18.2 + .8988X$
4. $y = 14.1 + .9673X$
5. $y = -4.3 + 1.3172X$
6. Plot 1, deep soil site; plot 2, hillside plot.

Appendix, Table 8a. Apparent digestibilities for Trial 1, percent.

Substrates Fed	Dry Matter	Cellulose	Crude Protein	Crude Fiber	Digestible Energy	Crude Fat	NFE	TDN	Metabolizable Energy [1]
Alfalfa Hay (chopped)									
Lamb 1	58.3	58.3	68.5	38.1	56.9	42.0	68.7	56.3	2144
Lamb 2	54.1	55.2	65.2	31.0	53.1	26.2	66.1	53.4	2124
Mean	56.2 ± 3.0	56.7 ± 2.2	66.8 ± 2.3	34.6 ± 2.4	55.0 ± 2.7	34.1 ± 11.2	67.4 ± 1.8	55.0 ± 1.8	2134 ± 0.4
Alfalfa (78%) + Sagebrush (22%)									
Lamb 3	55.7	54.4	62.4	36.1	54.2	50.6	64.1	54.3	2041
Lamb 4	55.7	54.2	62.3	38.4	53.7	51.9	64.1	55.0	2009
Mean	55.7 ± 0.0	54.3 ± 0.1	62.4 ± 0.1	37.2 ± 1.6	53.9 ± 0.3	51.2 ± 0.9	64.1 ± 0.0	54.7 ± 0.5	2025 ± 0.7
Sagebrush (by Difference)									
Lamb 3	54.1	44.6	32.5	43.1	51.3	69.2	54.2	44.8	
Lamb 4	53.7	43.3	31.9	55.6	49.2	71.8	54.3	47.5	
Mean	53.9 ± 0.3	44.0 ± 0.9	32.2 ± 0.4	49.4 ± 8.8	50.3 ± 1.5	70.5 ± 1.8	54.3 ± 0.1	46.2 ± 1.9	

[1] Kilocalories per kilogram intake, calculated as described by Cook and Associates (32, p. 579-590).

Appendix, Table 8b. Apparent digestibilities for Trial 2, percent.

Substrates Fed	Dry Matter	Cellulose	Protein	Crude Fiber	Energy	Crude Fat [1]	NFE
Alfalfa (green chop)							
Lamb 1	61.6	69.3	77.2	50.9	64.5		72.0
Lamb 2	62.3	69.3	77.9	54.2	65.5		72.2
Lamb 3	63.6	69.5	78.3	54.2	65.4		71.7
Lamb 4	63.4	72.4	77.4	48.7	64.9		74.2
Mean	62.7 ± .9	70.1 ± 1.5	77.7 ± .5	52.0 ± 2.7	65.1 ± .5		72.5 ± 1.1
Alfalfa (65%) + Sagebrush (35.0%)							
Lamb 5	61.3	68.2	75.9	49.6	64.9	51.5	64.9
Lamb 6	63.6	67.8	77.9	48.0	65.0	50.2	70.5
Lamb 7	57.5	62.1	71.7	34.7	57.3	42.9	67.6
Lamb 8	59.5	60.4	73.1	42.5	59.8	42.4	67.8
Mean	60.5 ± 2.6	64.6 ± 4.0	74.6 ± 2.8	43.7 ± 6.7	61.8 ± 3.8	46.8 ± 4.8	67.6 ± 2.5
Sagebrush (by Difference)							
Lamb 5	58.7	65.3	66.8	45.9	64.6		54.3
Lamb 6	65.3	63.6	78.7	40.6	65.0		67.1
Lamb 7	48.1	50.5	41.3	8.9	44.7		60.8
Lamb 8	53.5	45.9	49.1	27.6	50.8		61.2
Mean	56.4 ± 7.3	56.3 ± 9.6	59.0 ± 16.9	30.8 ± 16.5	56.3 ± 10.2		60.9 ± 5.2
Mixed Grass Hay (MGH)							
Lamb 9	61.5	68.1	42.8	59.5	59.4	65.8	68.8
Lamb 10	62.1	68.2	44.6	63.6	60.1	66.0	67.5
Lamb 11	64.5	70.4	51.7	64.5	62.3	66.2	69.5
Lamb 12	62.7	69.4	44.0	63.1	60.1	65.6	68.7
Mean	62.6 ± 1.3	69.0 ± 1.1	45.8 ± 4.0	62.7 ± 2.2	60.5 ± 1.3	65.9 ± 0.3	68.6 ± 0.8
MGH (87%) + Sagebrush (13%)							
Lamb 13	61.7	67.6	47.2	58.3	60.4	71.8	68.6
Lamb 14	60.7	65.2	51.6	57.2	58.4	68.0	66.5
Mean	61.2 ± 0.7	66.4 ± 1.7	49.4 ± 3.1	57.8 ± 0.8	59.4 ± 1.4	69.9 ± 2.7	67.6 ± 1.5
Sagebrush (by Difference)							
Lamb 13	55.3	56.7	54.5	16.3	60.9	85.7	68.3
Lamb 14	48.7	36.8	81.3	7.6	46.9	72.7	54.5
Mean	52.0 ± 4.7	46.7 ± 14.1	67.9 ± 19.0	12.0 ± 6.2	53.9 ± 9.9	79.2 ± 9.2	61.4 ± 9.8

[1] Crude fat digestibility was not determined for Alfalfa (green chop)

Appendix, Table 8c. Apparent digestibilities for Trial 3, percent.

Substrates Fed [1]	Dry Matter	Cellulose	Crude Protein	Crude Fiber	Digestible Energy	Crude Fat	NFE
Alfalfa Hay (pelleted)							
Lamb 1	62	59	75	45	62	39	72
Lamb 2	60	56	72	42	60	36	71
Lamb 3	59	55	72	40	60	42	70
Lamb 4	57	50	70	36	57	31	69
Mean	59.5 ± 2.1	55.0 ± 3.7	72.3 ± 3.5	40.8 ± 3.8	59.8 ± 2.1	37 ± 4.7	70.5 ± 1.3
Alfalfa (51%) + Sagebrush (49%)							
Lamb 1	58.0	57.5	67.7	32.7	53.8	61.2	66.8
Lamb 2	55.2	54.9	63.3	34.0	54.5	61.1	62.3
Mean	56.6 ± 2.0	56.2 ± 1.8	65.5 ± 3.1	33.4 ± .9	54.2 ± .5	61.2 ± 0.1	64.6 ± 3.2
Sagebrush by Difference							
Lamb 1	56.0	60.9	55.8	18.8	46.7	68.7	63.3
Lamb 2	51.7	54.9	48.0	26.5	50.5	65.9	57.3
Mean	53.9 ± 3.0	57.9 ± 4.2	51.9 ± 5.5	22.7 ± 5.4	48.6 ± 2.7	67.3 ± 2.0	60.3 ± 4.2

[1] Sagebrush was forced into the rumen via the fistula.