



The in vitro digestibility and utilization of Big Sagebrush and Black Sagebrush  
by Karl David Striby

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in  
Range Science

Montana State University

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Abstract:

The relative forage quality of 4 *Artemisia* taxa was evaluated as reflected by in vitro organic matter digestibility (IVOMD), crude terpenoid content, and forage utilization. Taxa included *A. nova*, *A. tridentata vaseyana*, *A. t. wyomingensis*, and *A. jt. tridentata*.

Current years growth was collected from each taxon on January 1, February 15, and April 1, 1981. Collections from *A. t. vaseyana* and *A. t. wyomingensis* were segregated into lightly and heavily grazed plants. Crude terpenoids on the foliage epidermis were extracted with chloroform and quantified. Extracted and intact (fresh) foliage were ground in liquid nitrogen and frozen until in vitro digestion. IVOMD was determined using mule deer, sheep, and steer rumen inocula. A total of 1569 plants from the 4 taxa were examined for degree of hedging from combined deer and elk utilization in the spring of 1981. Utilization assessment indicated big game preference for the taxa.

Order of increasing digestibility among intact taxa was *A. nova*, *A. t. vaseyana*, *A. t. wyomingensis* and *A. t. tridentata*. IVOMD generally increased from January to February to April. Fewer and contrasting differences in IVOMD were evident for extracted foliage among taxa and dates. *A. t. vaseyana* was consistently less digestible than *A. nova* which was generally less digestible than *A. t. wyomingensis* and *A. jt. tridentata*. Extracted foliage was an average 13.2% more digestible than intact foliage. All inocula had similar digestive efficiency.

*A. t. vaseyana* contained the lowest crude terpenoid level, *A. nova* and *A. t. wyomingensis* contained intermediate levels, and *A. t. tridentata* contained the highest level. Crude terpenoid concentrations were greater on January 1 and February 15 than on April 1. Non-volatile crude terpenoids decreased IVOMD through microbial inhibition and/or resistance to digestion. The crude terpenoid concentration was negatively associated with IVOMD within each taxon, however, compositional differences might account for the variability in IVOMD among the taxa. Even though non-volatile crude terpenoids decreased IVOMD, digestibility remained high relative to other forages. Variation in IVOMD and crude terpenoid concentration was not related to utilization differences. Increasing order of preference appeared to be *A. t. tridentata*, *A. nova*, *A. t. vaseyana*, and *A. t. wyomingensis*. Lightly and heavily grazed plants of either *A. t. vaseyana* or *A. t. wyomingensis* were digested equally well and contained similar crude terpenoid levels. Therefore, apparent animal grazing preference was not influenced by either digestibility or crude terpenoid concentration.

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November 27, 1985  
Date

Carl L. Hambolt  
Chairperson, Graduate Committee

Approval for the Major Department

November 27, 1985  
Date

Arthur E. Rinde  
Head, Major Department

Approval for the College of Graduate Studies

12-3-85  
Date

M. Malne  
Graduate Dean

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## ABSTRACT

The relative forage quality of 4 Artemisia taxa was evaluated as reflected by in vitro organic matter digestibility (IVOMD), crude terpenoid content, and forage utilization. Taxa included A. nova, A. tridentata vaseyana, A.t. wyomingensis, and A.t. tridentata. Current years growth was collected from each taxon on January 1, February 15, and April 1, 1981. Collections from A.t. vaseyana and A.t. wyomingensis were segregated into lightly and heavily grazed plants. Crude terpenoids on the foliage epidermis were extracted with chloroform and quantified. Extracted and intact (fresh) foliage were ground in liquid nitrogen and frozen until in vitro digestion. IVOMD was determined using mule deer, sheep, and steer rumen inocula. A total of 1569 plants from the 4 taxa were examined for degree of hedging from combined deer and elk utilization in the spring of 1981. Utilization assessment indicated big game preference for the taxa.

Order of increasing digestibility among intact taxa was A. nova, A.t. vaseyana, A.t. wyomingensis and A.t. tridentata. IVOMD generally increased from January to February to April. Fewer and contrasting differences in IVOMD were evident for extracted foliage among taxa and dates. A.t. vaseyana was consistently less digestible than A. nova which was generally less digestible than A.t. wyomingensis and A.t. tridentata. Extracted foliage was an average 13.2% more digestible than intact foliage. All inocula had similar digestive efficiency. A.t. vaseyana contained the lowest crude terpenoid level, A. nova and A.t. wyomingensis contained intermediate levels, and A.t. tridentata contained the highest level. Crude terpenoid concentrations were greater on January 1 and February 15 than on April 1. Non-volatile crude terpenoids decreased IVOMD through microbial inhibition and/or resistance to digestion. The crude terpenoid concentration was negatively associated with IVOMD within each taxon, however, compositional differences might account for the variability in IVOMD among the taxa. Even though non-volatile crude terpenoids decreased IVOMD, digestibility remained high relative to other forages. Variation in IVOMD and crude terpenoid concentration was not related to utilization differences. Increasing order of preference appeared to be A.t. tridentata, A. nova, A.t. vaseyana, and A.t. wyomingensis. Lightly and heavily grazed plants of either A.t. vaseyana or A.t. wyomingensis were digested equally well and contained similar crude terpenoid levels. Therefore, apparent animal grazing preference was not influenced by either digestibility or crude terpenoid concentration.

## INTRODUCTION

The widespread existence of the woody sagebrushes (subgenus Tri-tatae of the genus Artemisia) in western North America has a significant impact on the ecology and economics of rangeland resources (Beetle 1960, Gifford et al. 1979, Blaisdell et al. 1982). In recent years many investigators have agreed that members of this group cannot be placed into uniform taxonomic and management categories. The assorted taxa are valuable or undesirable in different degrees and have important site and management implications (Beetle 1970, Morris et al. 1976, McArthur 1979). The need to more accurately categorize sagebrush taxa for the purpose of improving resource management was summarized by A. A. Beetle who said in 1977,

"It is no longer fashionable to refer in a general way to sagebrush....This wide variety in ecological adaptation, distribution and significance reflects important differences in site potential and consequently in management technique. Knowledge of the ecological characteristics of the species, and in many instances the subspecies of sagebrush will be an important tool to the range manager."

The great diversity of the sagebrushes, their vast distribution and the dichotomous nature of their value, justifies intense efforts to understand their beneficial uses.

Shrubs are indispensable in the diets of many wild and domestic herbivores (Dietz 1972). Yet, less is known about the forage quality of shrubs than other forage classes occurring on rangelands (Rittenhouse and Vavra 1979).

The relative acceptability and nutritive value of the various sagebrush taxa are major components of their diverse ecological profiles

(Beetle and Johnson 1982). The utilization of sagebrush in the diets of livestock and big game animals varies from no use to heavy use on winter ranges, where other forage species may be limited (Urness 1979). During the winter when range animals may be under considerable nutritional stress, the nutritive value of available forages is of critical importance (Ward 1971, Wallmo et al. 1977). Sagebrush is relatively high in digestible protein, phosphorous and carotene when compared to alternate forage species during the winter (Cook et al. 1954). However, the essential oils and other secondary metabolic products of sagebrush are suspected to influence the preference, digestibility and intake of these shrubs by browsing animals (Nagy 1979, Kelsey et al. 1983).

Knowledge of the factors affecting the forage quality of the sagebrushes can influence management decisions on sagebrush ranges. Range management practices such as grazing systems, sagebrush control techniques and reseeding on big game ranges warrant site specific decisions that may benefit by considering the forage value of the particular sagebrush species or subspecies at hand (Beetle and Johnson 1982, Morris et al. 1976, Blaisdell et al. 1982).

Big sagebrush (Artemisia tridentata Nutt.) and black sagebrush (Artemisia nova Nelson) are two species that are widely distributed on western rangelands. Relative to other sagebrush species, big sagebrush ranks first in total acreage covered, being a dominant or subdominant on approximately 58,632,000 ha (226,374 square miles) (Beetle 1960). It is represented by three subspecies; basin (ssp. tridentata), mountain (ssp. vaseyana (Rydb.) Beetle), and Wyoming

(ssp. wyomingensis Beetle and Young) big sagebrushes. Black sagebrush ranks third in total acreage, occupying approximately 11,215,000 ha (43,300 square miles) (Beetle 1960). Together, these 4 taxa comprise over 70 percent of the total sagebrush acreage in the 11 western states (Beetle 1960, Beetle and Young 1965). Their significance to the environment and to range managers matches their extensive distribution.

This study was initiated in order to further the understanding of the forage quality and the unique chemistry of black sagebrush and the 3 subspecies of big sagebrush. Objectives were as follows:

1. Compare in vitro organic matter digestibility (IVOMD) of 4 sagebrush taxa during winter as an indication of relative nutritive value.
2. Distinguish forage utilization differences among sagebrush taxa on big game winter range as an indication of animal grazing preference.
3. Determine the concentration of crude terpenoids in the sagebrush taxa during winter.
4. Evaluate the influence of crude terpenoid concentration on the IVOMD of sagebrush.
5. Investigate the influence of digestibility and crude terpenoid concentration on the relative forage utilization of the sagebrush taxa.

## LITERATURE REVIEW

Factors of Forage Quality

Range research techniques which measure the quantity and quality of vegetation draw from several fields of specialization. Disciplines such as agronomy, ecology and animal husbandry are often utilized, while highly specialized fields such as plant physiology and animal physiology have made major contributions (Subcommittee on Range Research Methods 1962). Each science helps unravel the many interrelationships found in biological systems.

Mott (1973) identified 2 biological systems basic to the evaluation of forages. The environment-plant system and the plant-animal system were used to describe the quantity and quality of forages produced per unit of land, labor and capital. Both systems determined the total yield of animal products (meat, milk, wool, etc.) per unit area. Forage quality, in toto, could be measured by animal production response, providing animal potential was held constant and forage was the sole source of food.

Animal and range scientists agree that the quality of a forage depends on its nutrient content, the digestibility of the nutrients, the metabolizability of the digested nutrients and the amount the animal will consume (Cook and Harris 1968, Dietz 1970, Barnes 1973, Church 1977, Kothmann 1980). The 2 basic components of forage quality as described by Mott (1973) are "forage nutritive value" and "forage consumed" (Figure 1). According to Mott, forage nutritive value is determined by the following 3 factors: (1) nutrient composition, (2)

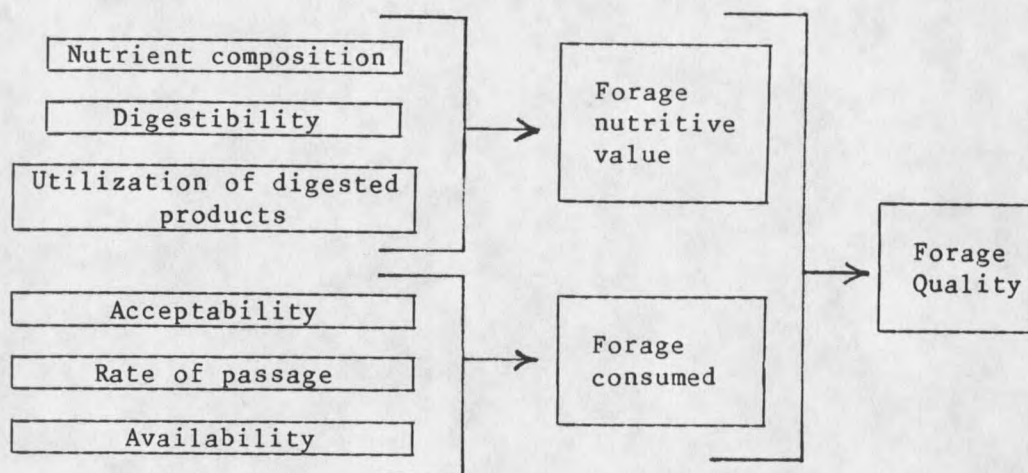


Figure 1. Diagrammatic representation of the factors of forage quality (adapted from Mott, 1973).

digestibility, and (3) utilization of digested products. Nutrient composition is dependent only on the plant and its environment. Digestibility and the efficiency of utilization of digested products are associated with both the plant and animal. When animal production potential and environmental effects are constant, forage consumed (also referred to as "voluntary intake") is determined by 3 factors as follows: (1) acceptability, (2) rate of passage, and (3) availability. Acceptability is described by the readiness with which a forage is selected and eaten. Rate of passage through the digestive tract is a function of forage fiber mass and rate of digestion. Availability refers to the amount of forage available for consumption. Each factor of forage consumption depends on the characteristics of both plant and animal.

Mott's (1973) description of forage quality considers environment and plant and animal parameters which are fundamental to optimum output of domestic livestock products. Terms commonly used to express overall

forage quality, such as "digestible energy consumption" or "rate of consumption of digestible dry matter" or "digestible organic matter intake", encompass the concepts of both forage nutritive value and forage consumed. These terms are recognized to be of prime importance in determining animal performance on pasture and range settings (Crampton 1957, Mott and Moore 1970, Rittenhouse and Vavra 1979). Currently, voluntary intake is considered as the more critical of the 2 components of forage quality in determining animal production potential (Ingalls et al. 1965, Mott and Moore 1970, Rittenhouse and Vavra 1979). While these principles are directly applicable to maximizing agricultural production, they are also relevant to the maintenance of healthy, productive wildlife populations (Dietz 1970, Wallmo et al. 1977).

The factors of forage quality pertinent to this study included digestibility and acceptability. A brief description of these factors and their importance follows.

#### Digestibility

The performance of all ruminant species utilizing range forage is, to a great extent, determined by the ability of the rumen microbes to efficiently utilize the nutrients in the forage consumed (Dietz 1970). Determination of forage digestibility has long been recognized as necessary to the assessment of forage nutritive value. In discussing nutrition of wild ruminants, Atwood (1948) stated that the gross composition of a forage alone was not capable of preventing starvation. Routine feed analyses were not always reliable as positive indicators

of nutritive value and such analyses must be supplemented by digestibility data. Blaxter et al. (1961) recorded a 100% increase in sheep weight gain when digestible dry matter of sheep diets increased from 50 to 55%. Digestibility is the most commonly used measure of forage nutritive value (Van Soest 1973). It is unlikely that a technique to assess nutritive value could be developed that did not take digestibility into account (Riewe and Lippke 1970).

An important nutritive element provided by forages in the diet of ruminants is energy (Reid et al. 1959, Cook 1970). Feeding standards for all animals are based, in part, on energy requirements (Church 1977). Total digestible nutrients (TDN) and digestible energy (DE) are measures commonly used to express energy needs. Digestible dry matter (DDM), digestible organic matter (DOM), TDN, and DE are highly correlated and these are often regarded as comparable measures of forage nutritive value (Swift 1957, Heaney and Pigden 1963, Reid et al. 1959, Rittenhouse et al. 1971, Van Soest 1973).

### Acceptability

Arnold (1964) asserted that an understanding of the mechanisms involved in forage selection and ingestion by ruminants are basic to our knowledge of their nutritional needs and productivity. Yet, the principles underlying forage acceptability are very complex and have long been in need of further clarification (Cook 1953, Hanley 1982). The foraging animal experiences a stimulus-response chain of events in food selection as follows: recognition of the food; movement to the food; appraisal; initial eating; cessation of eating. Food selection

may occur anywhere along the above chain and is controlled by a number of complex mechanisms (Heady 1975).

Palatability and preference are 2 different concepts that are inseparable in food selectivity (Heady 1975, Kothmann 1980). Heady (1964, 1975) defined palatability as plant characteristics which stimulate a selective grazing response by animals, and preference as animal reactions that facilitate food selection. Although neither forages nor plants, per se, exhibit preference, "forage preference" indicates a proportional choice among 2 or more forages by the grazing animal (Heady 1964). Forage palatability and animal preference are important factors governing range utilization and they represent valuable tools in formulating grazing management practices, stocking rates and pasture mixes (Subcommittee on Range Research Methods 1962, Malechek and Leinweber 1972).

There seems to be no universally accepted, specific definition for the term "palatability" since in the past it has been variously used to describe forage and animal attributes ranging from tastiness to voluntary intake (Martin 1970, 1978). In contrast to Heady's definition, the Range Term Glossary Committee (1974) defined palatability as "the relish with which a particular species or plant part is consumed by an animal." To further confound the subject, palatability and preference are some times used interchangeably (Heady 1964, 1975). Since it has been subjected to such wide interpretation, some authors have reported that the use of the term "palatability" in the discussion of the concepts operating in forage selectivity tends to embrace some degree of ambiguity (Mott 1959, McClymont 1967, Van Soest 1973, Stoddart, Smith

and Box 1975). In this thesis, the terms palatability and preference follow Heady's definitions.

According to Heady (1975), palatability factors are those characteristics of plants that are recognized by the animal senses of touch, smell and taste. They may stimulate a selective response of either acceptance or rejection. Major palatability factors are nutrient composition, growth stage, external plant form, associated feed elements and possibly digestibility. These, in turn, can be affected by season of use, weather patterns, soils and topography (Heady 1975).

In the past, palatability studies have placed much emphasis on nutritive values rather than the research of palatability, per se, resulting in a disproportionate concentration on composition of nutritive elements (proximate analysis, NDF, ADF, etc) (Heady 1964). Other palatability factors such as plant parts, plant growth stages, past grazing use, climate, topography, soil moisture and fertility have been examined mainly through their influence on nutrient composition (Heady 1964). No completely consistent relationship between any of the mentioned palatability factors and animal preference has been found, as many observations are the result of association rather than cause and effect (Martin 1970, Heady 1975). Many of the nutritive components that have been extensively researched, such as crude protein, crude fiber and lignin, etc., are hardly recognized by their smell or taste, while other compounds not measured by the usual nutritional analyses are responsible for stimulating these senses (Heady 1975). Within plant species similar in gross physical form and chemical

characteristics, selection may be based on subtle chemical differences affecting smell and taste (McClymont 1967).

While the extent of palatability influence on voluntary intake is not completely understood, many researchers agree that these factors are related (Van Soest 1973, Milchunas et al. 1978, Martin 1978). Some authors contend that forage palatability directly governs rate and total intake of forage (Jones 1952, Tribe 1952, Hurd and Blaser 1962, Dietz 1970). Others have reported that there is no evidence to indicate that highly preferred forages increase total intake, yet provision of unpalatable forages may reduce it (Van Soest 1965, McClymont 1967). Arnold (1964) maintained that animals can adapt to different grazing situations and consume as much of an unpalatable forage as a highly preferred one. A major question to answer when considering the extent to which palatability influences intake is whether the animal will consume enough of an unpalatable forage to reach either rumen capacity or energy balance (Martin 1970).

Many factors besides palatability affect food selection. Physiological and psychological conditions internal to the animal such as perception through the 5 senses, breeding, pregnancy, lactation, growth, fear, excitement, rumen fill and hunger influence animal behavior and thus preference (Heady 1975). Environmental factors such as climate, soil, topography and vegetation have a direct bearing on animal behavior and therefore influence food preference (Heady 1975). Evolutionary factors such as kind of animal and innate instincts differ markedly between animal species thereby altering diet selection. Learned behavior such as previous grazing experience can affect animal

food preferences (Heady 1975). This complex of stimulus-response mechanisms is not well understood for grazing ruminant animals (Heady 1975).

#### The In Vitro Rumen Fermentation Procedure

The in vivo digestibility trial is a time consuming and expensive procedure for estimating forage nutritive value (Barnes 1965). At least 3 animals and large amounts of forage are required to attain precise results (Heaney 1970). With unpalatable feeds or wild animals, feed intake can be a problem (Smith 1950, Bissel et al. 1955, Dietz et al. 1962, Milchunas et al. 1978, Striby et al. 1983). When a food item must be mixed with an acceptable base feed, the resultant digestibility by difference of the food item is sometimes confounded by interaction with the base feed (Dietz et al. 1962, Schneider and Flatt 1975, Milchunas et al. 1978, Striby et al. 1983).

The in vitro rumen fermentation procedure was designed to circumvent these difficulties. Because of its simplicity, speed, precision and economy, this procedure is widely used to estimate the digestibility of forages by wild and domestic ruminants (Pearson 1970). Numerous in vitro digestion techniques have been developed since its first recorded use by Pigden and Bell in 1955 (Barnes 1966). Of these, the two-stage technique of Tilley and Terry (1963) has the highest correlation and lowest standard error of the estimate for prediction of in vivo dry matter digestibility (Barnes 1965, 1966). Therefore, it is the method of choice used by many researchers for assessment of forage nutritive value (Oh et al. 1966, Van Soest 1967, Mott and Moore 1970).

Harris 1970, Pearson 1970, Urness et al. 1977, Milchunas and Baker 1982).

In vitro digestibility has been mainly reported as digestible cellulose or digestible dry matter (Pearson 1970). Cellulose digestibility, alone, is not necessarily a good predictor of forage nutritive value because cellulose is not characteristic of the whole forage dry matter (Barnes 1965, Van Soest 1967). In vitro dry matter digestibility is more often used because it is simpler to determine analytically and subject to less variability than in vitro cellulose digestibility (Johnson 1970). In vitro organic matter digestion is sometimes used to exclude error associated with indigestible ash which is principally composed of silica (Jones and Handreck 1967, Van Soest 1968, Milchunas et al. 1978).

In vitro digestion procedures have limited application in that results are not absolute measurements of in vivo parameters such as DDM, DE and TDN (Johnson 1966). Also, procedures are subject to significant variation inherent in biological systems and resulting from inconsistent laboratory methods (Johnson 1966). These limitations should be recognized before interpreting and applying in vitro results to the animal, across laboratories and across some localities (Johnson 1966).

Generally, in vitro digestibility data has 2 applications in the assessment of forage nutritive value. In unaltered form, it can be used as a relative ranking of forage digestibility (Johnson 1966). If in vitro data is to be used in establishing animal feeding practices or

nutritional requirements on the range, in vitro digestibility must first be correlated to in vivo digestibility (Johnson 1966). This is accomplished by determining the in vivo digestibility of standard forages by the ruminant species being studied. Standard forage samples, chosen to represent foods having a wide range of digestibility, are then included in subsequent in vitro digestion trials. Once uniform regression lines have been established between the in vivo and in vitro digestibilities of the standards, the in vitro technique can be used to predict the in vivo nutritive values of other forages (Johnson 1966). This is not necessary when only the relative ranking of forages is desired (Barnes 1965) which was an objective of this study.

Barnes (1967) found that the average in vitro digestible dry matter for 3 forages ranged from 38.7 to 53.3% among 5 laboratories using different methods. This variation was approximately reduced by half after employing identical methods (Barnes 1968). Barnes concluded that a standard procedure and consistent handling of inocula and each substrate reduces variation among laboratories and among runs and within runs.

The rumen fluid inoculum is the major source of variation in the in vitro system (Johnson 1966). Diet quality affects the abundance, diversity and activity of the microbial population in the rumen (Van Soest 1983), however there are conflicting reports regarding the effect of the diet of the inoculum donor on in vitro digestion. Differences in the precision and accuracy of in vitro digestion due to the diet donor have been demonstrated thereby implying that the inoculum donor should be fed the same forage to be evaluated, or a standard forage of

known in vivo digestibility (Warner 1956, Asplund et al. 1958, Church and Peterson 1960, Hungate 1960, Taylor et al. 1960, Van Dyne 1962, Bezeau 1965, Pearson 1970, Ward 1971, Nelson et al. 1972, Robbins et al. 1975, Horton et al. 1980). Yet some researchers have reported little or no variability in in vitro digestion due to the diet of rumen inocula donors (Quicke et al. 1959, Scales et al. 1974, NikKhah and Tribe 1977, Pederson and Welch 1982, Welch et al. 1983b). Different ruminant species do not digest all forages with equal efficiency (Van Soest 1983). This leads to the assumption that rumen inocula donor species might affect in vitro digestion thus requiring species-specific evaluations (Milchunas et al. 1978). As with the influence of diet, reports concerning effects of animal species on the precision and accuracy of in vitro digestion are inconsistent. Significant differences in extent and variability of in vitro digestion of some foods have been observed when inocula donors of different species were maintained on similar diets (Warner 1956, Asplund et al. 1958, Hungate et al. 1960, Ward 1971, Scales et al. 1974, Robbins et al. 1975, Palmer et al. 1976, Horton et al. 1980, Blankenship et al. 1982). Conversely, others have reported no significant in vitro digestion differences attributable to inoculum donor species (Van Dyne 1964, Le Fevre and Kamstra 1960, Welch 1983b). Other confounding evidence is that supplied by Troelsen and Hanel (1966), Ruggiero and Whelan (1976) and Milchunas et al. (1982), who found that the digestive capacity of rumen inoculum from the same animal, on the same feed can vary from one day to the next. Palmer et al. (1976) and Palmer and Cowan (1980) successfully predicted in vivo digestion by white-tailed deer with in vitro

estimates using rumen fluid inocula from Jersey cows maintained on forages other than those being evaluated. Troelson and Hanel (1966), Bryant and Robinson (1968) and Milchunas et al. (1982) conclude differences in the digestive capacities of rumen inocula are the result of sampling time after feeding and diet composition. Milchunas (1982) further suggests that it does not matter what species of rumen inoculum donor is used as long as it correlates well with the in vivo digestion of the particular animal species being studied.

#### The Forage Quality of Sagebrush

The food value of sagebrush, like other forage species, is influenced by the factors of forage quality as outlined by Mott (1973). However, unlike many forage species, the sagebrushes produce secondary metabolic compounds including the so-called "essential" or "volatile oils" that are believed to affect such factors as acceptability, digestibility and metabolizability (Cook et al. 1954, Nagy 1979, Kelsey et al. 1983). A review of the availability, acceptability and nutrient composition of sagebrush is followed by descriptions of its secondary metabolic products, and digestibility.

#### Availability and Acceptability to Ruminants

Because of its vast distribution and abundance, sagebrush is highly available for consumption by wildlife and domestic range animals (McArthur et al. 1979, Beetle and Johnson 1982). Sagebrush-grass vegetation is one of the largest, if not the largest, range ecosystem in the western United States (Blaisdell et al. 1982). The 20 or so sagebrush taxa comprising the subgenus Tridentatae inhabit approximately

108,782,000 ha (420,000 square miles) in 11 western states (McArthur et al. 1981, Beetle and Johnson 1982). Sagebrush species occur as climax shrubs in a variety of biomes (Gifford et al. 1979). The range of big sagebrush alone extends from near sea level to near tree line on approximately 58,632,000 ha (226,374 square miles) (Beetle 1960). It is the most common and widespread shrub in western North America (McArthur et al. 1979). Black sagebrush is the third most common sagebrush species being distributed over approximately 11,215,000 ha (43,000 square miles) in 10 western states (Beetle 1960).

Elk, domestic sheep, mule deer and pronghorn antelope, respectively, have been shown to be moderate to heavy consumers of sagebrush. Whereas pronghorn eat considerable amounts of sagebrush on a year-round basis (Sundstrom et al. 1973), sagebrush use by most ruminants occurs mainly during winter when herbaceous forage species are dormant and in limited supply or are covered by snow (Laycock 1967, Carpenter et al. 1979, Houston 1982). Kufeld et al. (1973) evaluated 99 food habits studies of the Rocky Mountain mule deer to determine the relative importance of forage plants as reflected by their consumption. Studies that referred to combined deer and elk use, or "game use" were excluded. In reference to big sagebrush, they listed 47 studies reporting heavy consumption during winter, 30 studies reporting heavy consumption during spring and 17 studies that cited moderate consumption during fall. In a similar evaluation of 60 food habits studies of the Rocky Mountain elk, Nelson and Leege (1982) listed 9 studies that reported moderate use of big sagebrush during winter and 4 studies that reported moderate use of big sagebrush during fall. Cook and Harris

(1954) reported that the diets of domestic sheep grazing Utah desert winter ranges of mixed flora contained 17% black sagebrush and 5% big sagebrush over a 5-year period. Laycock (1967) concluded that three-tip sagebrush (Artemisia tripartita Rydb.) and other shrubs constitute an important part of sheep diets and heavy late fall grazing is a practical method to control sagebrush. Similarly, Frischknecht and Harris (1973) concluded that sheep eat large amounts of big sagebrush in late fall and can control it on seeded cattle range. Narjisse (1981) reported that experienced sheep ate substantial quantities of basin big sagebrush during June and moderate amounts during August and November, while goats refused to eat it at these times. Generally, sagebrush consumption by cattle is low (Frischknecht and Harris 1973, Cook and Harris 1968, Blaisdell et al. 1982).

#### Nutrient Composition

During winter, sagebrush is relatively high in essential nutrients when compared to other forages. Grasses, forbs and deciduous shrubs become tough and dry, losing their nutritive value with advancing maturity, while sagebrush remains comparatively green and succulent (Dietz et al. 1958, Cook 1972). In assessing the nutrient content of important deer browse plants in southwestern Colorado, Dietz (1958) found that big sagebrush contained the highest levels of protein, phosphorous and carotene, and the lowest levels of crude fiber of the 5 species analyzed during late winter. Dietz concluded that big sagebrush is of outstanding significance in the management of deer winter range since it was the only browse species that substantially exceeded

minimum amounts of nutrients required by deer. Cook et al. (1954) evaluated the nutritive value of winter forage plants on ranges of western Utah and reported that black sagebrush and big sagebrush were decidedly higher in protein, phosphorous and carotene than grasses, which were superior only in energy. Cook concluded that black sagebrush and big sagebrush are valuable forage plants for domestic sheep on winter ranges and furnish adequate amounts of the important nutrients for pregnant ewes except in the case of energy.

#### Secondary Metabolic Products

The aromatic and bitter qualities of sagebrush have long been suspected to influence its forage value. Early records suggest that, because of its pungent nature, sagebrush was not relished by some animals and was possibly toxic (Bailey 1869, Pammel 1911). In reference to Artemisia tridentata, McCreary (1927) stated "Unfortunately the palatability of the leaves is lowered by the presence of a bitter principle and of nearly 3% volatile oil which gives them a bitter pungent taste." In 1931 McCreary further reported that there was little fat in the exceptionally high ether extract of big sagebrush, most of which was probably terpene-like compounds of low digestibility.

Sagebrush foliage is rich with a wide variety of secondary metabolic products having aromatic and bitter properties. As McCreary surmised in 1931, terpenoids are present in high concentrations. Substantial quantities of both monoterpenes and sesquiterpene lactones are present (Kelsey and Shafizadeh 1980, Welch and McArthur 1981), as well as lower concentrations of 2 classes of phenolics, the coumarins

(Shafizadeh and Melnikoff 1970) and flavonoids (Rodriguez et al. 1972). Highly volatile, non-terpenoid hydrocarbons such as acetone and methacrolein are also present (Scholl et al. 1977, Kelsey et al. 1983). In addition to these secondary products, cuticular waxes cover leaf surfaces (Thomas 1976, Kelsey et al. 1982). Each class of compounds has unique physical, chemical and biological properties that govern its isolation and analysis and may influence the forage quality of sagebrush (Kelsey et al. 1983). A brief description of these properties follow in Table 1.

Numerous studies have been conducted in an effort to clarify the relationships between the unique chemistry of sagebrush and its forage value. Most investigations have concentrated on the influence of the essential oils on digestibility and palatability. Conclusions drawn from these studies have been in some cases inconclusive and conflicting. Reports indicate that the essential oils of sagebrush kill rumen microorganisms, reduce microbial digestion of forage (Nagy et al. 1964, Nagy and Tengerdy 1968), reduce appetite (Nagy et al. 1964) and reduce palatability (Cook et al. 1954, Nagy and Tengerdy 1968, Sheehy 1975, Narjisse 1981), and increase both plant toxicity (Cook et al. 1954, Johnson et al. 1976) and non-metabolizable energy content (Cook et al. 1952). Conversely, others have found little or no net effect of the essential oils on sagebrush digestibility (Welch and Pederson 1981, White et al. 1982b, Cluff et al. 1982) or palatability (Scholl et al. 1977, Welch et al. 1983, White et al. 1982a).

Technically the essential oils are the volatile steam distillable components that give plants their characteristic odor (Harborne 1973).

Table 1. Properties of secondary metabolic products of sagebrush.

Class of compound	Percent foliage dry wgt	Physical state	Volatility	Sensory perception	Biological activity	References <sup>1</sup>
Monoterpenes	0.5-9.0	liquid	high	aromatic pungent	Antimicrobial Animal toxin Feeding deterrent Phytotoxic	1,2,3,4,5,6, 7,8,9,13
Sesquiterpene lactones	2.3-3.5	solid	low	bitter	Allergenic Antimicrobial Animal toxin Antifungal Cytotoxic Feeding deterrent <sup>2</sup> Phytotoxic	6,7,10,11 12,13,14,15 16,17,18,19 20,21
Phenolics (coumarins & flavinoids)	0.01-2.1	solid	low	astringent bitter sweet	Animal toxin <sup>2</sup> Cytotoxic <sup>2</sup> Feeding attractant Feeding deterrent <sup>2</sup> Phytotoxic	22,23,24,25, 26,27,28,29 30
Cuticular waxes & misc. compounds	3.8-10.2 <sup>3</sup>	solid	low	----	Limited digestibility	31
Highly volatile non-terpenoids (i.e. acetone, methacrolein)	unknown	liquid	very high	pungent	Possible feeding deterrent Mucosa irritant	1,32

Table 1. (continued)

<sup>1</sup>References:

- |                                  |                                     |
|----------------------------------|-------------------------------------|
| 1. Kelsey et al. (1983)          | 17. Picman and Towers (1983)        |
| 2. Sneva et al. (1983)           | 18. Wisdom et al. (1983)            |
| 3. Guenther (1948)               | 19. Picman (1984)                   |
| 4. Buttkus et al. (1977)         | 20. McCahon et al. (1973)           |
| 5. Nagy and Tengerdy (1968)      | 21. Amo and Anaya (1978)            |
| 6. Mabry and Gill (1979)         | 22. Harborne (1979)                 |
| 7. Kelsey et al. (1984)          | 23. Harborne (1982)                 |
| 8. Narjisse (1981)               | 24. Hanks et al. (1973)             |
| 9. Asplund (1968)                | 25. Stevens and McArthur (1974)     |
| 10. Kelsey and Shafizadeh (1980) | 26. Francis (1973)                  |
| 11. Shafizadeh et al. (1971)     | 27. Rice (1974)                     |
| 12. Kelsey (1974)                | 28. Shafizadeh and Melnikoff (1970) |
| 13. Rodriguez et al. (1976)      | 29. Rodriguez et al. (1972)         |
| 14. Mitchell et al. (1970)       | 30. Brown (1973)                    |
| 15. Mitchell and Dupuis (1971)   | 31. Martin and Juniper (1970)       |
| 16. Mitchell and Epstein (1974)  | 32. Lewis and Tatken (1980)         |

<sup>2</sup>The biological activities of many of the sesquiterpene lactones and phenolics specific to sagebrush species have yet to be tested. Activities cited here apply to related compounds isolated from other plant species.

<sup>3</sup>The range of values for percent cuticular waxes and miscellaneous compounds was approximated by subtracting minimum and maximum amounts of other secondary products from the respective minimum and maximum amounts of crude terpenoids cited in Kelsey et al. (1982) (ie. min. crude terpenoids-6.6% for vaseyana, max. crude terpenoids-24.8% for tridentata).

Many essential oils, including those of sagebrush, consist largely of liquid terpenoids (Guenther 1948, Nagy 1966). The monoterpenes are the most volatile and most abundant terpenoids found in many plant species, along with lesser amounts of sesquiterpenes and diterpenes (Guenther 1948, Tyler et al. 1981). For the purpose of this discussion, "sesquiterpenes" refers to the non-lactonized hydrocarbons ( $C_{15}H_{24}$ - $C_{15}H_{18}$ ) and related alcohols, aldehydes and ketones (Sutherland and Park 1967). Like other plant species, the terpenoid fraction of sagebrush essential oils is largely composed of monoterpenes (Scholl et al. 1977, Kelsey and Shafizadeh 1980, Welch and McArthur 1981); the literature suggests that sagebrush volatile oils do not contain significant amounts of sesquiterpenes or diterpenes. This author found only 1 reference to sesquiterpenes or diterpenes that have been isolated and positively identified from sagebrush volatile oils. Buttkus et al. (1977) reported that 2 sesquiterpenes and 4 diterpenes together comprises less than 1% of the steam distillable terpenoids in big sagebrush. The chemical and physical properties of the sesquiterpene lactones categorically separate them from the sesquiterpenes. Because they are crystalline solids with high melting points, the sesquiterpene lactones do not readily volatilize and generally are seldom found in steam distilled essential oils (Sutherland and Park 1967). Sagebrush foliage contains substantial quantities of sesquiterpene lactones (Kelsey and Shafizadeh 1980, Kelsey et al. 1982). However, none are found in the steam distilled essential oils of sagebrush. Likewise, the crystalline phenolics and cuticular waxes are absent from sagebrush volatile oils (Kelsey 1984, personal communication). At the other end of the

volatility scale are the non-terpenoid organic compounds such as acetone and methacrolein. Because of their extreme volatility, these compounds are lost during steam distillation and are absent in steam distilled essential oils (Kelsey et al. 1983).

The substitution of solvent extraction in place of steam distillation as the extracting process has in recent years revealed an array of secondary compounds in sagebrush tissues. The coumarins and sesquiterpene lactones were isolated, identified and recognized as taxonomic markers for shrubby Artemisia species (Young 1965, Winward and Tisdale 1969, Shafizadeh and Melnikoff 1970, Brunner 1972, Hanks et al. 1973, Kelsey et al. 1976). Using a chloroform solvent, Kelsey et al. (1982) separated the external components of sagebrush foliage from the internal constituents (Figure 2). The terpenoids and possibly the phenolics are present within glandular trichomes located on leaf surfaces that are coated with cuticular waxes (Kelsey and Shafizadeh 1980, Kelsey et al. 1983). These external components are readily available for solvent extraction. Collectively, the extract of external components constitute the "crude terpenoids" (Kelsey et al. 1982). The internal constituents are cell wall polymers, proteins, nonstructural carbohydrates and lipids.

The volatile, non-terpenoid organic compounds such as acetone and methacrolein are probably located in the glandular trichomes (Kelsey 1984, personal communication). Because of their extreme volatility, they are lost during the extraction process and therefore are not found in the crude terpenoids (Kelsey 1984, personal communication).

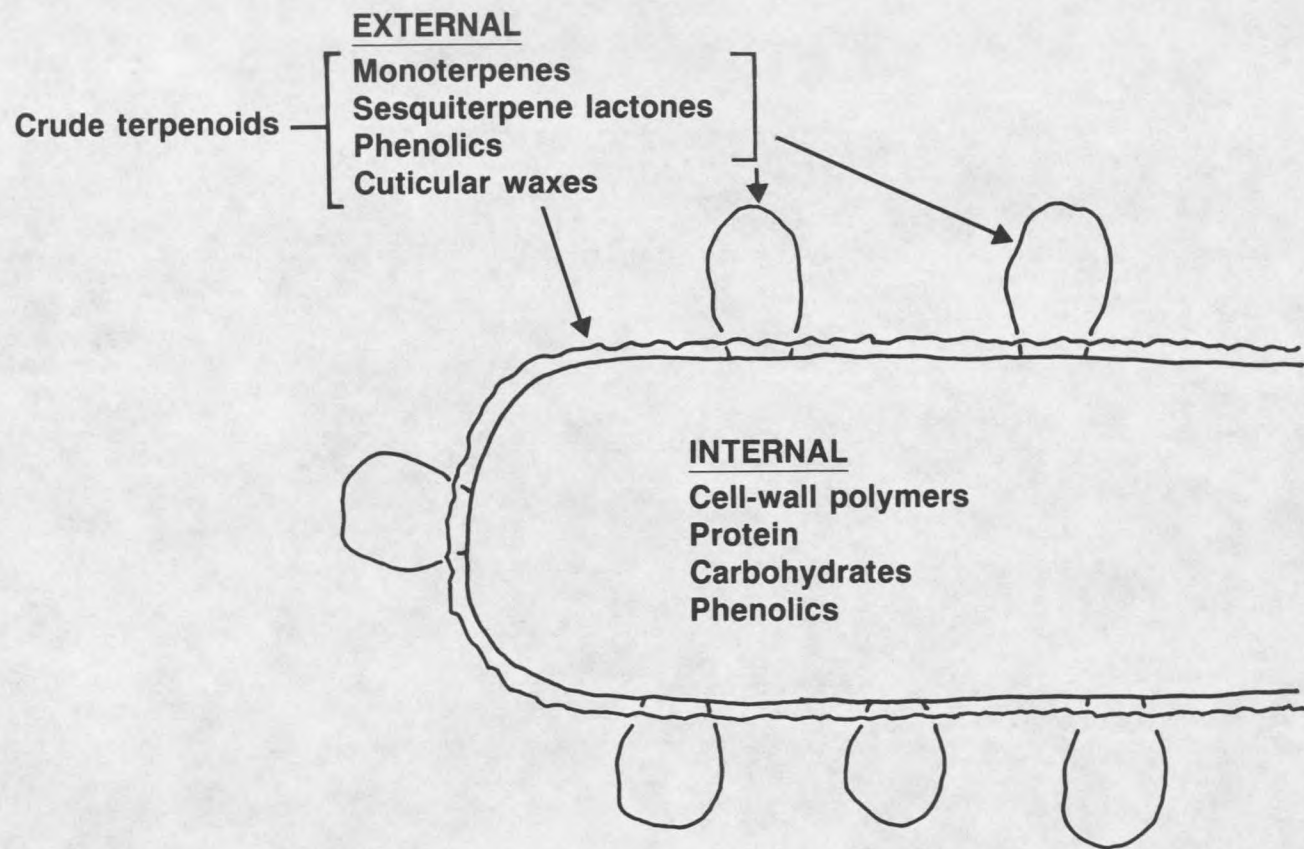


Figure 2. Internal and external components of sagebrush foliage. Leaf shown in cross section with protruding glandular trichomes. Phenolics may be internal and external components. Volatile non-terpenoid hydrocarbons (ie. acetone, methacrolein) probably occur in glandular trichomes.

When touched by feeding herbivores, the glandular trichomes readily burst and release their contents. Thus, a highly concentrated zone of volatile and non-volatile compounds is exposed to the olfactory and gustatory senses of the herbivore. In effect, certain of these compounds might independently or synergistically influence animal selection and digestion (Kelsey et al. 1983).

The literature indicates that the interaction of the secondary metabolites of sagebrush with its forage quality has primarily been evaluated in regard to the influence of the monoterpenes. The total impact of the secondary products of sagebrush on digestibility and forage selection has not been fully researched since the possible influence of the aforementioned classes of compounds not included in the essential oils have been largely overlooked (Kelsey et al. 1983).

#### Sagebrush Digestibility

Numerous reports on the DDM and TDN contents of big sagebrush indicate that it is moderately to highly digestible relative to alfalfa and winter range forages (Tables 2 and 3). Comparative TDN values determined for alfalfa by Smith (1952), Bissell et al. (1955), Dietz et al. (1962) and Smith (1963) were 64.7%, 65.5%, 54.3% and 59.5% respectively. Smith (1957) discovered that the TDN level in big sagebrush (Table 4) exceeded the amounts found in 7 other deer browse species by at least 12.6%. Ward (1971) determined that big sagebrush had the highest in vitro dry matter digestibility (IVDMD) of 11 winter range forages including 5 grasses, 5 shrubs and 1 forb (Table 5). Urness et al. (1977) ranked the IVDMD of big sagebrush (Table 5) second highest

Table 2. In vivo digestibility of big sagebrush.

Reference	Subspecies Designation	Test Animal	Ave DDM	Ave TDN	DE Kcal/kg	ME
Smith (1950, 1957)	<u>typica</u> <sup>1</sup>	deer	67.1 <sup>2</sup>	78.1	----	----
Cook et al. (1952)	none	sheep	-----	43.4	1946	1130
Cook et al. (1954)	none	sheep	37.6	50.7	2304	1268
Bissell et al. (1955)	none	deer	48.9 <sup>2</sup>	55.9	2848 <sup>2</sup>	----
Dietz et al. (1962)	none	deer	49.6 <sup>2</sup>	58.9	----	----
Smith (1963)	<u>tridentata</u> <sup>3</sup>	sheep	54.5	55.9 <sup>2</sup>	2485	1636 <sup>2</sup>

<sup>1</sup> Subspecies typica from classification system of Hall and Clements (1923); synonymous with subsp. tridentata of Beetle (1960).

<sup>2</sup> Values calculated from author's reported data.

<sup>3</sup> From classification system of Beetle (1960) which did not recognize subsp. wyomingensis.

Table 3. In vitro digestibility of big sagebrush.

Reference	Subspecies Designation	Test Animal	Ave DDM
Smith (1963)	<u>tridentata</u> <sup>1</sup>	sheep	47.5
Ward (1971)	none	elk	52.3
	none	cow	52.3
Sheehy (1975)	<u>tridentata</u>	unspecified	56.8
	<u>vaseyana</u>	unspecified	53.6
	<u>wyomingensis</u>	unspecified	53.1
Urness et al. (1977)	<u>typica</u> <sup>2</sup>	deer	62.0
Wallmo et al. (1977)	none	deer	59.0
Narjisse (1981)	<u>tridentata</u>	sheep	50.0 <sup>3</sup>
	<u>tridentata</u>	goat	51.0 <sup>3</sup>
Welch and Pederson (1981)	<u>tridentata</u>	deer	62.1
	<u>vaseyana</u>	deer	53.2
	<u>wyomingensis</u>	deer	51.4
Kufeld et al. (1981)	combination <sup>4</sup>	cow	49.9
Pederson and Welch (1982)	none	deer	67.0

<sup>1</sup> From classification system of Beetle (1960) which did not recognize subsp. wyomingensis.

<sup>2</sup> Sagebrush remained from Smith (1950) study (Table 2).

<sup>3</sup> Digestible organic matter.

<sup>4</sup> Average of 3 major subspecies.

among 11 mule deer forages including 8 shrubs and 3 forbs. Alfalfa was the only forage having more IVDMD than big sagebrush out of a set of 4 native and 2 domestic forages tested by Pederson and Welch (1982). For black sagebrush, Cook et al. (1954) detected 38.6% DDM and 47.2% TDN while Sheehy (1975) reported 53.1% IVDMD.

A variety of digestibility techniques and animal species were used in compiling the data in Tables 2 and 3. Smith (1950, 1957) and Bissell et al. (1955) (Table 2) used single species diets while Dietz et al. (1962) and Smith, (1963) (Table 2) used the by-difference method whereby the diet consisted of sagebrush and another forage. Cook et al. (1952, 1954) (Table 2) used the lignin-ratio technique and allowed animals to graze single-species stands. All in vitro digestion trials (Table 3) utilized variations of the Tilley and Terry two-stage technique, except Smith (1963), who used a one-stage method. It is not the purpose of this review to evaluate the relative digestive efficiency of experimental techniques or animal species. However, it should be noted that many factors affect the digestibility of a particular plant species, including environmental conditions, stage of plant maturity, animal species, animal selectivity and nutritive balance of the ration (Cook et al. 1954). The digestibility of an individual plant species when eaten separately may be different than when other species are included in the diet (Cook et al. 1954, Dietz et al. 1962). Therefore, the establishment of exact digestion coefficients under controlled conditions may be only an approximation of the actual digestibility on mixed species ranges (Cook et al. 1954,

Dietz et al. 1962). These factors should be considered when interpreting the data in Tables 2 and 3.

Many genetic and environmental factors that affect shrub metabolism, morphology and nutrient content also affect the digestibility of shrub tissues (Short et al. 1972). The genetic, ecological and morphological diversity within the big sagebrush complex was not adequately characterized until the 3 major subspecies were described by Beetle and Young in 1965. Prior to this time, classification systems beyond the species category were incomplete or not used (Tables 2 and 3). Some workers have suggested that the secondary metabolic products of sagebrush might influence digestion (Nagy et al. 1964, Kelsey et al. 1983). The composition of nutrients and secondary products in big sagebrush are under genetic control (Shafizadeh and Melnikoff 1970, Kelsey et al. 1976, Welch and McArthur 1979a, 1981, Kelsey et al. 1982, 1983). However, the majority of more recent digestibility assessments have failed to make subspecies distinctions (Table 3). Sheehy (1975) measured the IVDMD of the 3 major subspecies of big sagebrush (Table 3), but statistical analysis was not possible because of inadequate sample size. The only substantial evaluation reporting digestibility of each subspecies was conducted by Welch and Pederson (1981). They found that A. t. subspecies tridentata had significantly greater IVDMD than either subspecies vaseyana or wyomingensis (Table 3). Kufeld et al. (1981) did not report subspecies differences (Table 3), but maintained that the variation in IVDMD among the subspecies, as reflected by coefficients of variation, appeared to be small enough to permit application of mean or median values.

Forage digestibility is directly affected by the changes in nutrient composition that parallel phenological development (Cook and Harris 1968, Short et al. 1972). If the secondary metabolic products of sagebrush influence digestion, then changes in their concentrations associated with phenological development (Smith 1963, Kelsey et al. 1982) might affect sagebrush digestibility. During the winter big sagebrush is physiologically dormant relative to the other seasons (Kelsey et al. 1982). However, fluctuations in the winter levels of crude protein, crude fiber, cellulose, essential oils and crude terpenoids have been shown to occur (Dietz et al. 1958, 1962, Smith 1963, Kelsey et al. 1982). Few studies have attempted to evaluate the effects of these compositional changes on sagebrush digestibility. Cook et al. (1954) examined the nutrient content and digestibility of big and black sagebrush during the fall and winter. Their results between dates were highly variable and without evidence of seasonal trends. In many cases, digestion trials were conducted several hundred miles apart, thus much of the variability in their data could have been attributed to site or even subspecies differences. Bissell et al. (1955) recorded little change in the nutrient composition or digestibility of big sagebrush from January to March, but noted that extreme differences in consumption of the straight sagebrush diet by deer tended to mask any real differences in digestibility. Smith (1963) monitored the nutrient composition, essential oil concentration and IVDMD of big sagebrush through out all seasons. Sagebrush digestibility was highest in the spring and winter when essential oil, crude fiber and cellulose levels were lowest. Ward (1971) reported highly

significant increases in the IVDMD of big sagebrush from November to March. A 10% decrease in IVDMD of big sagebrush from January to February was reported by Wallmo et al. (1977). Narjisse reported that the in vitro organic matter digestibility (IVOMD) and crude protein level in basin big sagebrush decreased from June to August and increased from August to November while corresponding monoterpene levels followed an opposite trend.

#### Influence of Essential Oils on the DE, TDN and DDM in Sagebrush

Cook et al. (1952) reported that sagebrush has a relatively high ether extract content, much of which is composed of essential oils. Normally, the ether extract content of a feed is largely composed of lipids which are important nutritionally as a source of energy (Church 1977). Lipids and essential oils have high heats of combustion, approximately 9.4-9.5 kcal/g (Church 1977, Weast and Astle 1980). Consequently, sagebrush gross energy content (GE) is relatively high. However, Cook et al. (1952) believed that the essential oils are non-nutritive substances that are polymerized in the liver or kidneys and excreted in the urine. This is especially relevant to TDN and DE determinations which measure nutrient and energy losses only in the feces. Cook et al. (1952) reasoned that TDN and DE would tend to be overestimated in sagebrush since the essential oils are not recovered in the feces and thus would appear to be absorbed and utilized as an energy source. For species containing large amounts of essential oils, metabolizable energy content was deemed a more appropriate index than either TDN or DE because it accounts for energy losses in the urine and

fermentation gases. They demonstrated this principle by showing that big and black sagebrush contained nearly as much TDN and DE as grasses and alfalfa, whereas ME levels were substantially lower in the sagebrush species.

However, the DE and ME levels in big sagebrush reported by Cook et al. (1952) are considerably less than those cited in other studies (Table 2). According to Bissell et al. (1955) and Smith (1963), DE levels in big sagebrush (Table 2) meet or exceed DE levels in many high quality pre-bloom and early-bloom forages reported by Church (1977). In these cases, energy losses from urine and fermentation gases of the same magnitude reported by Cook et al. (1952) would give big sagebrush more ME than 1130 kcal/kg. Indeed, ME values as high as 1690 kcal/kg, reported by Cook et al. (1954), and those from Smith (1963) (Table 2) are similar to ME values reported by Church (1977) for intermountain meadow grasses and mid-bloom prairie grasses. This suggests that while DE in big sagebrush may be overestimated as theorized by Cook et al. (1952), ME content may be comparable to ME levels found in other range forages.

Others have surmized that TDN and DDM reduction due to the influence of essential oils are not nearly as great as the energy losses reflected in ME. This reasoning stems from the fact that TDN is compiled from 4 nutrient fractions and essential oils are a part of only the ether extract fraction. Smith (1957) estimated that the TDN in juniper would be reduced from 63.5 to 60.8 if the essential oil content of 2.1% was subtracted from the ether extract fraction. Welch and McArthur (1979b) and Welch et al. (1982) estimated that the TDN in big

sagebrush would be reduced from 63.4% to 58.9% if the essential oil content is 2.0%. Although their reasoning is not clear, Welch and McArthur (1979b) adjusted for the influence of essential oils on IVDMD of sagebrush by subtracting essential oil concentration from IVDMD values. They concluded that even after correcting for essential oils, big sagebrush TDN and DDM content still ranked very high relative to other mule deer forages.

Cook et al. (1952) reported a 12.4-18.0% loss of energy (600-870 kcal/kg) in the urine for sagebrush species and an average 3.8% (160 kcal/kg) for grass species and alfalfa. The large difference in energy losses between sagebrush and the other species amounting to 440-710 kcal/kg (600-870 kcal/kg minus 160 kcal/kg) was attributed to the essential oils in sagebrush. Cook et al. (1952) did not measure essential oil content of sagebrush. Assuming that the sagebrush contained an average 2.0% essential oils (Nagy and Tengerdy 1968; Welch and McArthur 1981, Narjisse 1981, Kelsey et al. 1982), and monoterpenes contain an average 9500 kcal/kg (Weast and Astle 1980), then the sagebrush used by Cook et al. (1952) would have lost only about 190 kcal/kg from essential oil polymers excreted in the urine. However, considerable quantities of essential oils may be lost during ingestion, mastication, rumination and eructation (Welch and McArthur 1981, White et al. 1982b, Cluff et al. 1982). These processes would decrease the amount of essential oils available for absorption in the rumen meaning even less of the predicted 190 kcal/kg of energy would be passed in the urine. This is substantially less than the 440-710 kcal/kg that Cook et al. (1952) attributed to essential oil losses in the urine. They

believed that the essential oils were converted to complex polymers because volatile compounds could not be detected in the urine and the ether extract of the urine did not account for the increased calorific loss. Perhaps other high-energy, non-volatile substances, in addition to essential oils, are excreted in the urine. These substances might include unmetabolized secondary products such as sesquiterpene lactones, coumarins, flavonoids and cuticular compounds.

The conjectures of Cook et al. (1952), Smith (1957) and Welch and McArthur (1979b) that the TDN and IVDMD content of sagebrush is overestimated seem correct, but possibly for inaccurate reasons. Welch and Pederson (1981) demonstrated that oven-drying sagebrush at 100°C resulted in a total loss of essential oils (monoterpenes). Before determining ether extract, forage samples are routinely oven dried at 100°C (Association of Official Agricultural Chemists 1965). This procedure would inadvertently drive off the essential oils. Hence, they would not be represented in the ether extract content of the forage dry matter consumed by the animal during in vivo digestion trials. Consequently if standard procedures are followed, the digestible ether extract fraction of TDN would already exclude essential oils, thus making it unnecessary to subtract them from the ether extract content of the feed. Bissell et al. (1955) reported that oven dried sagebrush had an ether extract of 14% whereas sagebrush dried in a desiccator contained 21%. Sesquiterpene lactones, coumarins, flavonoids and cuticular compounds would be present in the ether extract fraction even after oven-drying samples because they have high boiling points and are not easily volatilized and many are ether-soluble. It would seem more

appropriate to subtract the contribution of these substances from the ether extract fraction in order to adjust TDN instead of the essential oils. However, the concentration of these compounds in the ether extract has not been determined. The concentration of sesquiterpene lactones, coumarins, flavonoids and cuticular compounds may range from 6-16% of sagebrush dry matter (Table 1). As is the case with essential oils, these compounds have a variety of biological activities, therefore, they probably are not metabolized by ruminants but are excreted in the feces and/or urine. However, unlike essential oils, these compounds may remain in the forage dry matter even after oven drying. In vivo and in vitro DDM are determined by subtracting the dry matter content of the feces, or forage residue, from the dry matter content of the forage input. If substantial quantities of lactones, phenolics and cuticular compounds are eliminated in the urine, they would appear to be digested thus overestimating in vivo DDM. A similar effect on IVDMD would occur if these compounds are lost during filtration and washing of the forage residue remaining in fermentation tubes.

The aforementioned theories concerning the nutritional role of secondary metabolic products may affect the correlation of DDM with TDN, DE and ME. The relationships among these factors in sagebrush species may be different from those normally found in other forage species. For example, the nearly 1:1 relationship between DDM and DE found in many forage species (Heaney and Pigden 1963, Rittenhouse et al. 1971) may not hold for sagebrush.

Influence of Essential Oils on Rumen Microbial Function

The literature contains numerous evaluations of the interaction between essential oils and rumen microorganisms. Basically, two conflicting theories have emerged. One theory maintains that the essential oils inhibit rumen microbial activity thereby reducing digestion, and if large amounts of sagebrush are consumed, the oils can become toxic. Animals can lessen deleterious effects by limiting consumption, and rumen microbes can adapt to the oils. Others contend that in nature, high levels of essential oils in the rumen are not attained, and effects on microbial digestion and toxicity problems are nil. Rumen microbes do not have to adapt to the oils.

Nagy et al. (1964) determined that big sagebrush essential oils have a broad spectrum of antibacterial properties, acting on both gram-positive and gram-negative organisms. In vitro studies of mule deer rumen microbial activity showed a definite depression of gas and volatile fatty acid (VFA) production due to the addition of sagebrush oils. They predicted that the levels of oils used might actually occur in the rumen if the diet consisted of 100% sagebrush. Digestion of cellulose broth by deer rumen bacteria decreased and was entirely inhibited as increasing amounts of oils were added. Nagy et al. (1964) concluded that cellulose digestion in deer would be slowed down slightly if the diet contains 15-30% sagebrush having 1-2% essential oils. Nagy et al. (1964) found that appetite and rumen motility ceased completely after 21 pounds of big sagebrush was introduced in 7 pound daily portions through the rumen fistula of a steer. VFA concentration in the steer rumen decreased from 123 mM/liter before receiving

sagebrush to 55 mM/liter on the third day of feeding. They suggested that with excessive sagebrush feeding, deer might experience the same reactions as the steer, although probably to a lesser degree; and, adaptation of rumen bacteria while animals are continually digesting sagebrush might be an important factor.

Nagy and Tengerdy (1967) showed that 4 species of aerobic bacteria varied significantly in their resistance to increasing amounts of essential oils. Furthermore, significant differences were found between the influence of the oils from big sagebrush and black sagebrush on 3 out of 4 bacteria species tested. However, they concluded that at least until more data were accumulated, the antibacterial action of the oils of the two plant species should be considered the same. Yet, because the current annual growth of big sagebrush contained approximately twice the amount of essential oils as black sagebrush, they suggested that in nature, big sagebrush may be a more potent antibacterial agent than black sagebrush.

Oh et al. (1967) studied sheep and deer rumen microbial activity as affected by various essential oils isolated from Douglas-fir needles. Microbial activity was measured in terms of fermentation gases produced in vitro. Two major classes of compounds isolated from the Douglas-fir essential oils, namely the monoterpene hydrocarbons and oxygenated monoterpenes, are commonly found in sagebrush. Of the 7 monoterpene hydrocarbons tested from fir needles, alpha-pinene, beta-pinene, myrcene, camphene and carene have been isolated from sagebrush essential oils by various workers (Kelsey 1983, Buttkus et al. 1977, Welch and McArthur 1981). All 5 of these compounds promoted slightly

or had no effect on deer microbial activity, whereas all promoted sheep rumen microbes, except carene, which inhibited activity. Three of the 7 oxygenated monoterpenes tested from fir needles, namely alpha-terpineol, terpinen-4-ol and fenchyl alcohol, have been isolated from sagebrush (Buttkus et al. 1977). All of these compounds inhibited deer and sheep rumen microbial activity. There were no net effects on rumen microbial activity from additions of lower levels of whole essential oils. However, as additions of whole oils increased, there was significant net reduction in microbial activity. The authors attributed these effects to interactions between promoting and inhibiting classes of compounds. Sheep and deer rumen microbes were affected differently by the various essential oils. Sheep microorganisms were promoted and inhibited to greater extents than were deer microbes. Deer rumen microbes unexposed to Douglas-fir were affected more than deer microbes exposed to fir. The authors assumed that the predominant species and numbers of rumen microbes in sheep and deer rumina differed according to their habitat, and the deer microbes subjected to Douglas-fir browse had become adapted to the inhibitory essential oils.

Nagy and Tengerdy (1968) reported that the essential oils of big sagebrush exerted marked antibacterial effect on both wild and captive mule deer rumen bacteria at concentrations greater than 16  $\mu$ l/10ml of rumen fluid nutrient broth (RFNB). The average colony count of rumen bacteria after 24 hours incubation with big sagebrush oils at the above concentration was  $8.57 \times 10^8$  for wild deer known to have been consuming considerable amounts of big sagebrush and  $2.22 \times 10^7$  for captive deer having no exposure to sagebrush. At an oil concentration of 18

ul/10ml RFNB bacterial counts for wild and captive deer were  $6.76 \times 10^8$  and  $5.77 \times 10^4$  respectively. At higher oil concentrations the bacterial counts for all deer were reduced to similar levels. Statistically significant differences were obtained when the averages of wild deer bacterial counts were compared with the averages of the captive deer. However, based on these seemingly large bacterial count differences, Nagy and Tengerdy concluded that reactions of rumen bacteria obtained from wild as well as captive deer were of the same magnitude, thus, no adaptation by the bacteria to the oils was apparent. They surmised that if deer rumen contents contain an average 15% dry matter and if the diet consisted totally of sagebrush with an oil content from 1-5%, then 10ml of rumen contents would contain from 15-75 ul of essential oils. Based on these figures they estimated that deer could safely tolerate a diet of approximately 50% sagebrush without ill effects. The possibility of considerable variation in either direction, dependent on various interactions between rumen and host animal, was recognized. Dietz and Nagy (1976) stated that small concentrations of essential oils do not significantly affect rumen microbes, whereas at higher concentrations there is pronounced inhibition. They demonstrated this point by increasing the amounts of sagebrush essential oils administered to a rumen fistulated goat receiving a standard pelleted ration. The goat went off feed when oil concentration reached approximately 15 ul per 10 ml of rumen fluid. They noted this level of oils was similar to the concentration cited by Nagy et al. (1964) that caused antimicrobial activity in deer. Based on the previous work of Nagy and Tengerdy (1967), Dietz and Nagy (1976) concluded that on a

gram per gram basis, black sagebrush essential oils were less inhibitory than big sagebrush oils.

Schwartz et al. (1980a) determined that the concentration and composition of essential oils varied considerably among Rocky Mountain (Juniperus scopulorum), Utah (J. ostersperma) and alligator (J. deppeana) junipers. They tested the influence of whole essential oils and oil fractions on microbial digestion of cellulose, starch and dry matter of juniper and alfalfa using in vitro techniques. Juniper oil fractions included monoterpene hydrocarbons and oxygenated monoterpenes common to sagebrush. Results indicated that oxygenated monoterpenes inhibit digestion of cellulose, starch and dry matter more than monoterpene hydrocarbons. Whole Utah juniper oils were most inhibitory to starch and dry matter digestion and contained the highest concentration of oxygenated monoterpenes. Whole alligator juniper oils were least inhibitory but did not contain the least amount of oxygenated monoterpenes. However they did contain the most monoterpene hydrocarbons. Levels of essential oils required to inhibit cellulose digestion (0.4-0.6 ul/ml cellulose broth) were lower than oil levels required to inhibit digestion of starch (1-5 ul/ml starch broth). Amounts of Rocky Mountain and alligator juniper oils required to inhibit digestion of alfalfa dry matter (0.7-1.3 ul/ml media) were lower than oil levels required to inhibit digestion of juniper dry matter (2.0-3.3 ul/ml media). These results coincide with the earlier findings of Smith (1963) who suggested that the inhibitory effect of essential oils at a given level may be specific to certain species of microorganisms which may or may not be required in the fermentation of a particular forage.

Sundstrom et al. (1973) noted that during winter sagebrush comprises as much as 90% of pronghorn antelope diets without producing ill effects. They proposed that antelope rumen microflora may be highly adapted to sagebrush. Furthermore, the relatively large size of the antelope's liver and kidneys compared to those of domestic sheep may be important adaptations that allow greater metabolism of essential oils and increased capacity to filter and excrete toxic substances.

Smith (1963) tested the effect of big sagebrush essential oils on in vitro rumen fermentation by adding increasing amounts of oils to Solka-floc (pure cellulose), alfalfa, mixed-grass hay and sagebrush substrates. The addition of 3 times the level of oils normally found in sagebrush prevented digestion of Solka-floc, decreased IVDMD of the mixed-grass hay and alfalfa, and had no effect on sagebrush digestibility. However, reductions in digestibility for mixed-grass hay and alfalfa were relatively small and Smith noted that quite satisfactory IVDMD was still obtained for the forages even at the unnaturally high levels of essential oils. Smith (1963) and Smith et al. (1966) concluded that there is a specific rather than general inhibitory effect of sagebrush essential oils on rumen microorganisms which is not of practical concern in the elimination of normal rumen function.

Ward (1971) found no harmful effects from the essential oils of big sagebrush and rubber rabbitbrush on elk and steer rumen protozoa which were periodically examined during in vitro digestibility trials. At the end of the digestion period there was a general decline in protozoa activity for all plant species tested.

Welch and Pederson (1981) evaluated the *in vitro* digestible dry matter of the 3 subspecies of big sagebrush (Table 3). Rumen inocula was taken from wild mule deer inhabiting sagebrush range in order to enhance the possibility that rumen microbes were adapted to sagebrush. Samples from 9 accessions of big sagebrush were ground in liquid nitrogen in order to minimize the escape of volatile oils. They found no relationship between total essential oil content and digestibility. However, camphor ( $r = -.75$ ) and camphene ( $r = -.75$ ) had significant negative influence on digestible dry matter. Camphor (an oxygenated monoterpene) accounted for about 25% of the variation in digestibility while camphene (a monoterpene hydrocarbon) accounted for less than 1%. Since the digestibility of sagebrush was relatively high, it was suggested that the impact of camphor and camphene on digestion was small. To ascertain the fate of monoterpenes during digestion trials, d-camphor and alpha-pinene were added to control tubes containing only buffer solution at 2.5 g/ml buffer. After the first incubation period 17.3% of the camphor and 100% of the alpha-pinene were lost from the control tubes. Camphor remaining in the tubes condensed as a ring above the buffer solution. The authors concluded that monoterpenoids are lost from the rumen and therefore do not significantly interact with the rumen microorganisms. They proposed 3 ways in which monoterpene levels may be reduced: (1) loss through mastication and rumination, (2) volatilization from body heat and eructation and (3) absorption and excretion in the urine.

Narjisse (1981) assessed the effects of monoterpenes on rumen microbial activity by infusing various concentrations of monoterpenes

directly into the rumens of anosmic sheep and goats. Concentrations approximated the range of levels that occur in quantities of sagebrush realistically consumed by the animals. After infusion, samples of rumen fluid were periodically taken and tested for fermentative ability and analyzed for monoterpenes. No significant differences in fermentative ability of rumen fluid samples were found among the various concentrations of monoterpenes at any length of time after infusion. Monoterpenes could not be detected in rumen fluid samples 4 hours after infusion. Narjisse suggested that monoterpenes may leave the rumen through rumination and eructation, excretion in the urine and metabolism. He rejected the hypothesis that monoterpenes depress rumen microbial activity in sheep and goats.

White et al. (1982b) observed that pygmy rabbit diets consisted of 97% sagebrush. They reported that the monoterpenoid content of stomach ingesta was 77% less than in the forage consumed. Using an airtight rabbit chamber, the authors demonstrated that a portion of the monoterpenes are volatilized during mastication and ingestion. Thus, adverse interactions between monoterpenes and microbes in the stomach and cecum were greatly reduced. In a similar study, Cluff et al. (1982) compared the monoterpenoid content of rumen ingesta from mule deer with levels in the diet. On the average, sagebrush, juniper and rabbitbrush comprised over half of the diet. On a dry matter basis, the monoterpenoid concentration in the rumen ingesta was 80% less than the concentration in the diet. They concluded that mastication, rumination, eructation, excretion in the urine and metabolism in the liver may

reduce the concentration of monoterpenes in the rumen to levels that do not interfere with microbial activity.

Pederson and Welch (1982) reported that mule deer rumen inocula that had not been exposed to big sagebrush or juniper essential oils, and mule deer inocula that had been exposed to big sagebrush oils, digested various forages, including big sagebrush, equally well. They concluded that rumen microorganisms do not have to adjust to essential oils.

## SITE DESCRIPTION

The study site was located on Travertine Flats 1.6 km (1 mile) north of Yellowstone National Park near Gardiner, Montana. The area, locally known for its travertine quarries, is composed of several basalt benches at the base of the Absoroka Mountains. The broadest of these encompass an area 0.8 - 1.6 km (0.5 - 1.0 miles) wide and 5 km (3 miles) long at an elevation of 1854 - 1902 m (6080 - 6240 feet). Most slopes have a southwest aspect.

A rainshadow formed by the surrounding mountains creates a dry climate. Annual precipitation is 356 - 381 mm (14 - 15 inches), half of which falls as snow (Farnes 1975).

Thermal springs, glacial drift and natural erosion have influenced the topography and soils of the area thus creating a mosaic of micro-sites and vegetation. Big sagebrush and black sagebrush dominate with bluebunch wheatgrass (Agropyron spicatum) and Idaho fescue (Festuca idahoensis) subdominants. So-called "cafeterias" exist where the 3 subspecies of big sagebrush and black sagebrush are found in close proximity. Cafeterias are comprised of habitat types as described by Mueggler and Stewart (1980) and refined by McNeal (1984).

The study site is part of the northern Yellowstone winter range. Depending on snow accumulations at higher elevations and winter severity, up to 5,000 elk and 400 mule deer migrate to the winter range from Yellowstone Park and the surrounding mountains (Houston 1982, McNeal 1984). Moderate to heavy use of sagebrush on the winter range by both elk and deer has been reported (Houston 1982, McNeal 1984).

## STUDY PROCEDURES

Taxa Identification

Identification of sagebrush taxa was based on several criteria. In the field, morphological characteristics (Morris et al. 1976, Winward 1980) were reinforced by examining the ultra-violet light fluorescence of alcohol and water leaf extracts (Winward and Tisdale 1969, Stevens and McArthur 1974). Subtle differences in the fragrance emitted by crushed leaves helped separate certain subspecies (Hanks et al. 1973, McArthur et al. 1979). Specimens of each taxon from the study site were sent to the University of Montana, Wood Chemistry Laboratory for further identification using thin-layer chromatography techniques (Kelsey et al. 1976, 1982).

Sagebrush Browse Collection

Browse material was collected from each of the 4 sagebrush taxa at the study site on January 1, February 15, and April 1, 1981. Collections from mountain and Wyoming big sagebrushes were subdivided into lightly and heavily browsed plants. Thus, 6 sagebrush categories were recognized (Table 4).

Four groups, each consisting of 5 plants, were selected from each sagebrush category giving a total of 120 plants as shown in Table 4. Selected plants were morphologically typical of taxon, mature, of ample size to allow 3 collections and were totally available for browsing. The plant groups were located and marked within stands that consisted of predominantly 1 sagebrush taxon to minimize the chance of collecting from hybrid plants.

Table 4. Plant grouping scheme for sagebrush browse collection.

Sagebrush category <sup>1</sup>	Plant groups per category	Plants per group <sup>2</sup>	Plants per category
nov	4	5	20
tri	4	5	20
vas-light	4	5	20
vas-heavy	4	5	20
wyo-light	4	5	20
wyo-heavy	4	5	20
Total plants clipped =			120

<sup>1</sup>nov = black sagebrush; tri = basin big sagebrush; vas-light, vas-heavy = mountain big sagebrush - lightly and heavily browsed, respectively; wyo-light, wyo-heavy = Wyoming big sagebrush - lightly and heavily browsed, respectively.

<sup>2</sup>The same 5 plants comprised a group for each collection.

Approximately 10 grams of live, current year leaf and stem growth was clipped from each of the 5 plants in a group and placed into a single 16 oz. NASCO Whirl-Pac plastic bag. The filled Whirl-Pacs were placed in a Thermos cooler with ice then transported to the laboratory freezer. The same 120 plants were clipped and grouped identically on each of the 3 dates.

#### Crude Terpenoid Extraction

In the laboratory, a composite sample of browse material was made from each sagebrush category. This was accomplished by combining representative subsamples of approximately 5g from each of the 4 Whirl-Pacs comprising a sagebrush category. The remaining material was returned to the freezer for subsequent in vitro digestion. The composite samples thus obtained were derived from 20 plants. Crude

terpenoid compounds were removed and quantified from each composite sample in duplicate using a rapid extraction technique with chloroform according to Kelsey et al. (1982). Excess chloroform was driven off the extracted plant materials by drying over-night in an oven at 65°C. They were then placed in Whirl-Pacs and frozen for in vitro digestion.

#### Preparation and Handling of In Vitro Digestion Samples

Effort was made to minimize the escape of volatile oils from intact (unextracted) sagebrush materials. Sample preparation and weighing was carried out in a refrigerated locker that was maintained at 3°C. Browse material was super-frozen in liquid nitrogen then ground using a motorized mortar and pestle. The ground samples were sealed in airtight plastic bottles and stored in a freezer maintained at -23°C. For consistency these same methods were used on extracted plant materials. A total of 90 sagebrush samples were prepared (Table 5). Dry matter and organic matter were determined in duplicate for each sample.

Before weighing samples for in vitro digestion, the sample bottles were removed from the freezer and allowed to equilibrate with the refrigerator locker temperature. Aliquots, weighed to 4 decimals, were adjusted so that approximately 0.5g of organic matter was placed in each in vitro digestion tube. Tubes were sealed with rubber stoppers fitted with Bunsen valves then returned to the freezer. In this manner samples were not exposed to room temperature until initiation of in vitro digestion. Prior to inoculation, tubes and samples were prewarmed in a water bath to 39°C.

Table 5. Number of intact<sup>1</sup> and extracted<sup>2</sup> sagebrush samples prepared for in vitro digestion<sup>3</sup>.

Sagebrush category	Collection date					
	1/1/81		2/15/81		4/1/81	
	Int	Ext	Int	Ext	Int	Ext
nov	4	1	4	1	4	1
tri	4	1	4	1	4	1
vas-light	4	1	4	1	4	1
vas-heavy	4	1	4	1	4	1
wyo-light	4	1	4	1	4	1
wyo-heavy	4	1	4	1	4	1

Total intact samples = 72; Total extracted samples = 18

<sup>1</sup>Crude terpenoids not removed.

<sup>2</sup>Crude terpenoids removed.

<sup>3</sup>One intact sample from each taxon group, and one composite extracted sample, at each collection date.

#### In Vitro Digestion Procedure

The Moore modification of the Tilley and Terry in vitro digestion technique (Harris 1970) was used to determine the in vitro organic matter digestibility (IVOMD) of the samples. Three in vitro digestion trials were conducted using rumen fluid inoculum from a wild mule deer, a domestic sheep and a steer.

On April 18, 1981, Montana Department of Fish, Wildlife and Parks personnel shot a healthy two-year old buck mule deer on the study site. The entire rumen and contents were removed and transported in a prewarmed container to a wet lab facility 4 miles away. Approximately 30 minutes elapsed between removal of the rumen and initiation of the in vitro trial. Single intact sagebrush samples and duplicate extracted

samples were tested. Western wheatgrass (Agropyron smithii Rydb.), alfalfa (Medicago sativa L.) and wheat straw (Triticum aestivium L.) were samples included for comparison.

A rumen fistulated sheep and a rumen fistulated steer provided rumen inoculum for the remaining 2 digestion trials conducted at the Montana State University Animal Nutrition Center. Both animals were maintained on mixed grass hay diets at least 2 weeks prior to collecting inoculum. Feed and water was withheld overnight before vacuum pumping rumen fluid directly from the rumen into a prewarmed container. In both trials, single intact sagebrush samples were tested, whereas extracted sagebrush samples were duplicated. Samples included for comparison were western wheatgrass and wheat straw.

#### Relative Utilization Assessment

Five areas where all 4 sagebrush taxa grew within close proximity to one another were located on the study site. Each area allowed browsing mule deer and elk to select among the sagebrush taxa in cafeteria fashion. Boundaries were arbitrarily assigned to each cafeteria so there were approximately equal amounts of each taxon. Size ranged from 0.5 to 1.0 ha (1.2 - 2.5 acres).

Approximately 75 plants from each sagebrush taxon were observed in each cafeteria for degree of hedging from past use. Plants were assigned to one of 3 form classes according to Cole (1958): zero to light hedging; moderate hedging; heavy to severe hedging. Plants were chosen for observation by objectively running 3 x 30.5m belt transects through stands consisting of predominately 1 sagebrush taxon to

minimize the chance of encountering hybrids. All plants within a belt transect that were at least 30cm tall (12 inches), or in the case of A. nova, at least 30cm in diameter, and available for browsing were observed.

### Statistical Analysis

Data were expressed as percent in vitro organic matter digestibility (IVOMD) and percent crude terpenoids on a moisture-free basis. Data from each digestion trial were analyzed separately. Analysis of variance (ANOVA) was used to evaluate a factorial set of classes. Factors were sagebrush categories (Table 4) and sagebrush collection dates (Table 5). A description of the statistical model appears in Appendix A. Error mean square terms used in ANOVA were constructed for extracted IVOMD data and crude terpenoid data by utilizing an estimation of plant to plant variation associated with intact IVOMD means (Appendix B). The protected least significant difference method was used to test IVOMD and crude terpenoid mean differences. A paired t-test was used to detect significant differences between intact and extracted IVOMD means of common taxon and date classes. Analysis of covariance (ANCOVA) was used to evaluate the influence of the covariate, crude terpenoid level, on the IVOMD of intact sagebrush. ANCOVA were conducted separately for each digestion trial. The distribution of shrubs among browse form classes was assessed with the Chi-square test. All analyses were conducted according to Snedecor and Cochran (1980).

## RESULTS

In Vitro Organic Matter Digestibility Trials

ANOV showed no significant differences ( $P < .05$ ) in IVOMD between lightly and heavily browsed mountain or Wyoming big sagebrush (Appendix C, Tables 8 and 9). Therefore, IVOMD values for light and heavy categories were averaged in further data analyses. Significant sources of variation revealed by ANOV were sagebrush taxon and collection date (Figures 3, 4, 5). There were no significant taxon x date interactions.

General trends in IVOMD among sagebrush taxa and collection dates and between intact and extracted sagebrush were nearly the same for all inocula sources (Figures 3, 4, 5). For the deer, sheep and steer, respectively IVOMD of intact sagebrush ranged from 37.6 - 63.4%, 41.5 - 59.6% and 42.4 - 58.4%; and IVOMD of extracted sagebrush ranged from 56.4 - 75.7%, 53.8 - 75.2% and 43.7 - 72.3%. The overall average IVOMD of intact sagebrush for the deer, sheep and steer was 50.2%, 51.1% and 51.5%, respectively; and extracted sagebrush was 63.5%, 66.0% and 60.1% respectively. Because of the limited number of digestion trials, it was not possible to statistically analyze differences between animal species. However, large differences in digestive ability between inocula donor species were infrequent (Figures 3, 4 and 5). The deer trial was conducted in a temporary field-laboratory using wild deer inoculum. These conditions did not permit the level of procedural consistency that was employed in the sheep and steer trials, and may

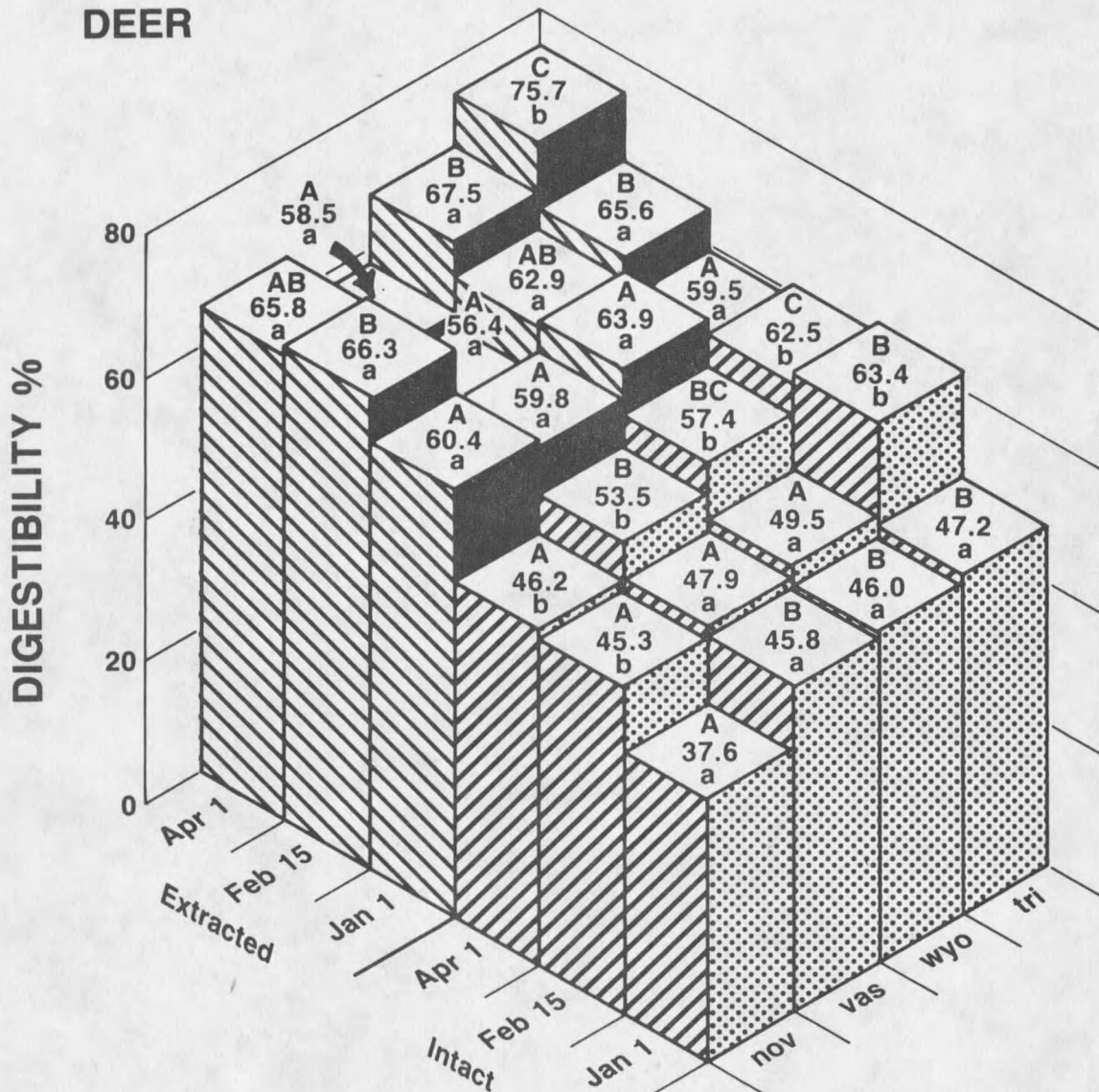


Figure 3. In vitro organic matter digestibility of 4 sagebrush taxa on 3 dates using deer inoculum. Crude terpenoids were intact and extracted. Taxa: nov= black sagebrush; vas, wyo, tri= mountain, Wyoming and basin big sagebrushes, respectively. Values among taxa on a given date with unlike capital letters are different ( $P < .05$ ). Values among dates for a given taxa with unlike small letters are different ( $P < .05$ ). Letter comparisons do not apply across extracted and intact data.

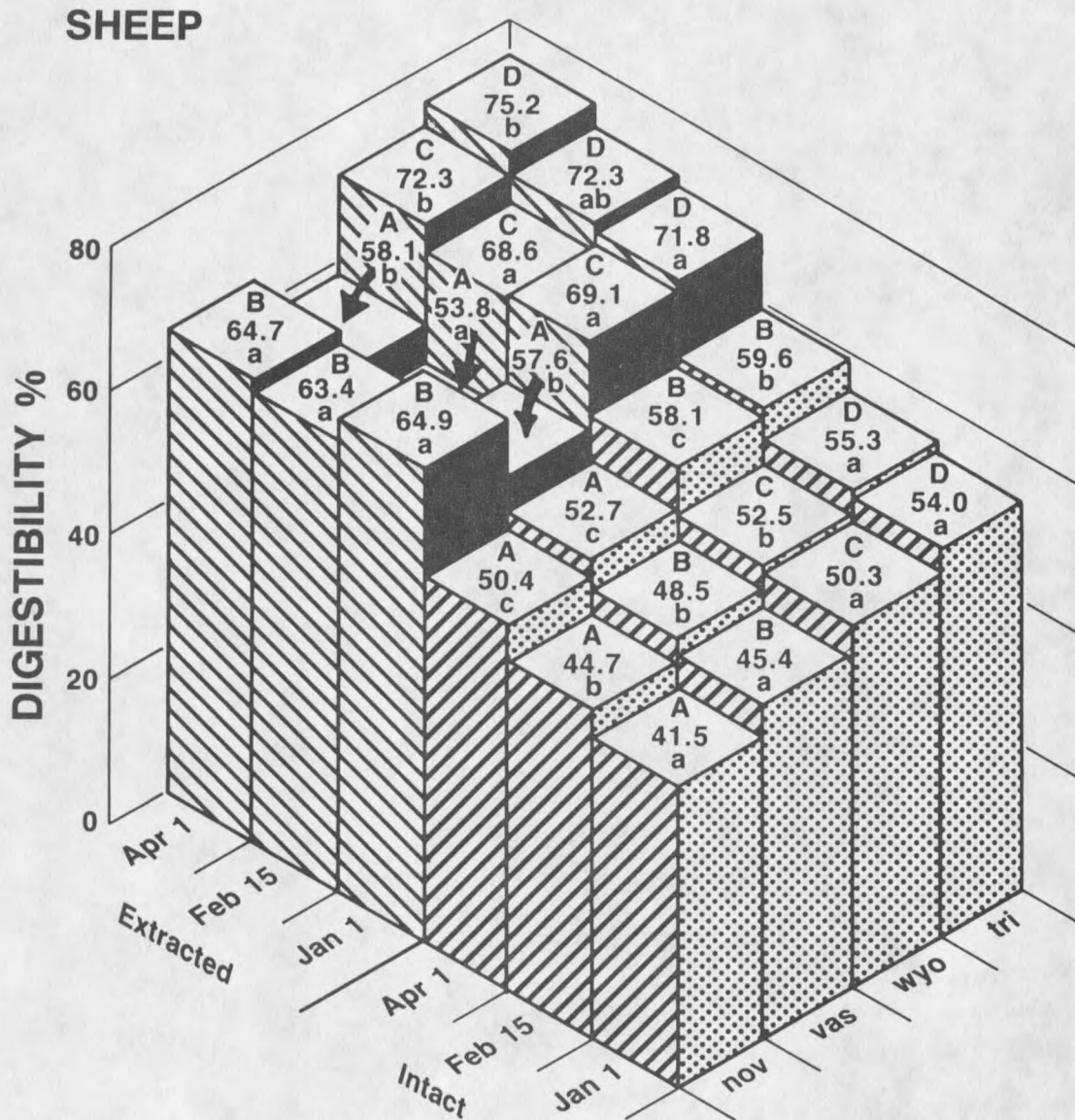


Figure 4. In vitro organic matter digestibility of 4 sagebrush taxa on 3 dates using sheep inoculum. Crude terpenoids were intact and extracted. Taxa: nov= black sagebrush; vas, wyo, tri= mountain, Wyoming and basin big sagebrushes, respectively. Values among taxa on a given date with unlike capital letters are different ( $P < .05$ ). Values among dates for a given taxon with unlike small letters are different ( $P < .05$ ). Letter comparisons do not apply across extracted and intact data.

## STEER

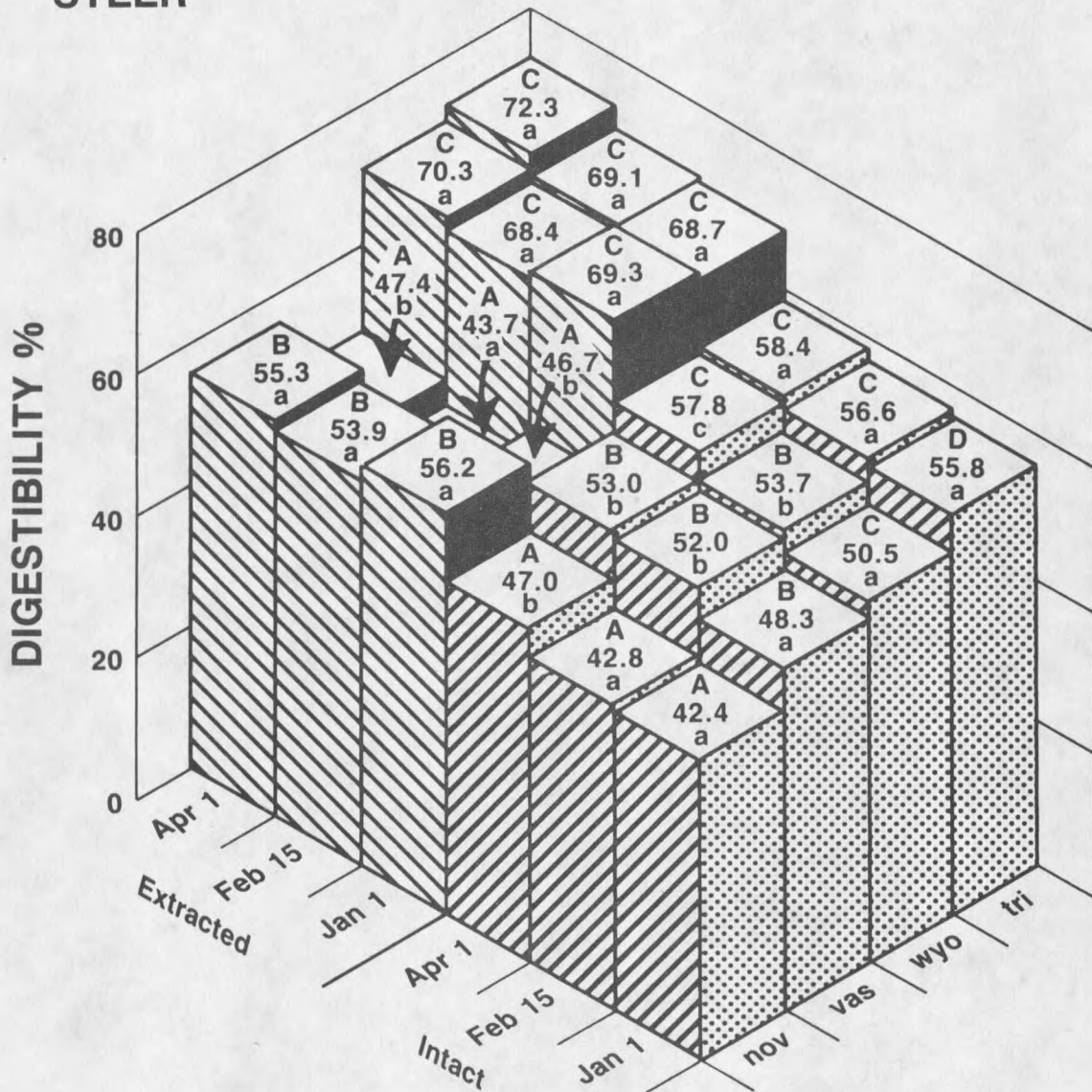


Figure 5. In vitro organic matter digestibility of 4 sagebrush taxa on 3 dates using steer inoculum. Crude terpenoids were intact and extracted. Taxa: nov= black sagebrush; vas, wyo, tri= mountain, Wyoming and basin big sagebrushes, respectively. Values among taxa on a given date with unlike capital letters are different ( $P < .05$ ). Values among dates for a given taxa with unlike small letters are different ( $P < .05$ ). Letter comparisons do not apply across extracted and intact data.

have contributed to the greater variability observed in the deer IVOMD data.

In vitro organic matter digestibility of western wheatgrass, alfalfa and wheat straw using the deer inoculum was 56.0%, 67.9% and 29.6%, respectively. Western wheatgrass and wheat straw included in the sheep trial had 54.0% and 34.1% IVOMD, respectively; and in the steer trial 50.3% and 32.6% IVOMD, respectively.

#### Intertaxon Digestibility

In all digestibility trials and on all collection dates, order of increasing digestibility among intact sagebrush was black sagebrush, mountain, Wyoming and basin big sagebrushes (Figures 3, 4 and 5). However, on any particular date, differences between taxa were not always significant. This was most evident for the deer which had the most variable data (Figure 3). On the other hand, when IVOMD was averaged for each taxon over all dates, most taxonomic differences were significant (Figures 6, 7 and 8). For deer, the digestibility of black sagebrush was significantly less than mountain and Wyoming big sagebrushes which were significantly less digestible than basin big sagebrush (Figure 6). There were significant IVOMD differences among all intact sagebrush taxa for the sheep and steer (Figures 7 and 8).

Order of increasing IVOMD among extracted sagebrush taxa differed from intact sagebrush (Figures 3, 4 and 5). Generally, there were fewer significant differences between taxa for all animals. In all trials, mountain big sagebrush had significantly less IVOMD than the other taxa (Figures 6, 7 and 8). Black sagebrush, Wyoming and basin big

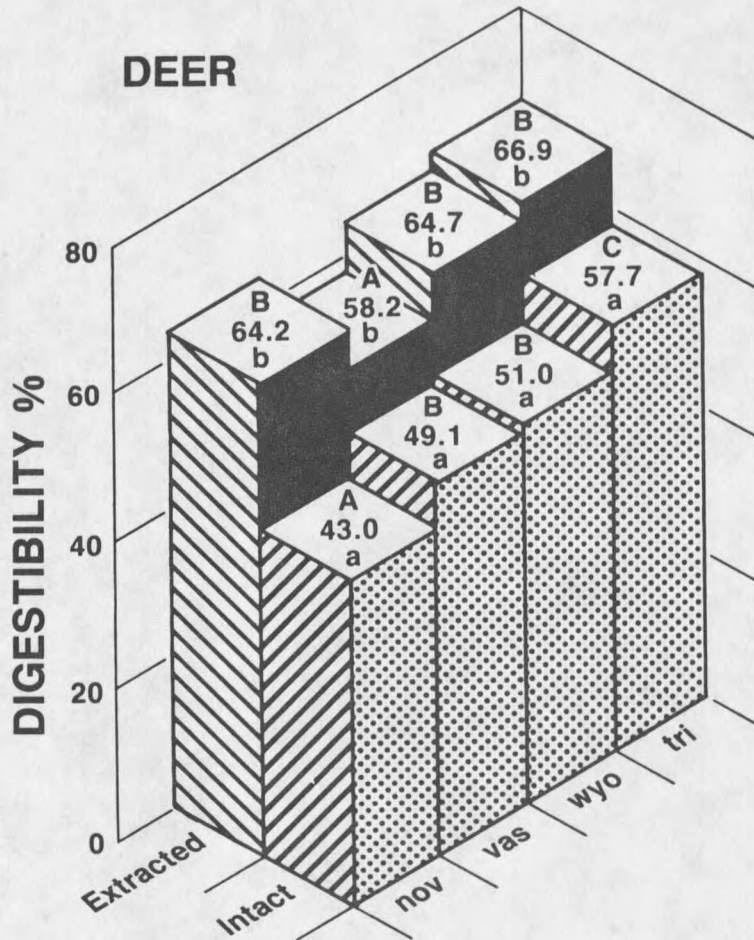


Figure 6. In vitro organic matter digestibility of 4 sagebrush taxa averaged for 3 dates (Jan. 1, Feb. 15, Apr. 1) using deer inoculum. Crude terpenoids were intact and extracted. Taxa: nov= black sagebrush; vas, wyo, tri= mountain, Wyoming and basin big sagebrushes, respectively. Extracted or intact values among taxa with unlike capital letters are significantly different ( $P < .05$ ). Values between extracted and intact of a given taxon with unlike small letters are significantly different ( $P < .05$ ).

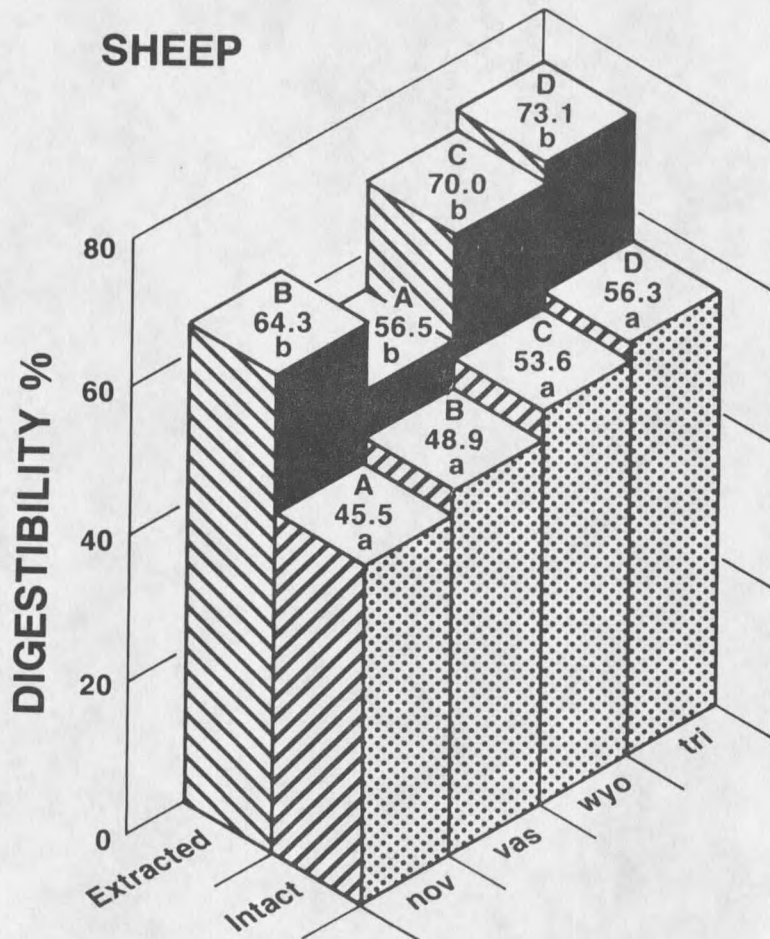


Figure 7. In vitro organic matter digestibility of 4 sagebrush taxa averaged for 3 dates (Jan. 1, Feb. 15, Apr. 1) using sheep inoculum. Crude terpenoids were intact and extracted. Taxa: nov= black sagebrush; vas, wyo, tri= mountain, Wyoming and basin big sagebrushes, respectively. Extracted or intact values among taxa with unlike capital letters are significantly different ( $P < .05$ ). Values between extracted and intact of a given taxon with unlike small letters are significantly different ( $P < .05$ ).

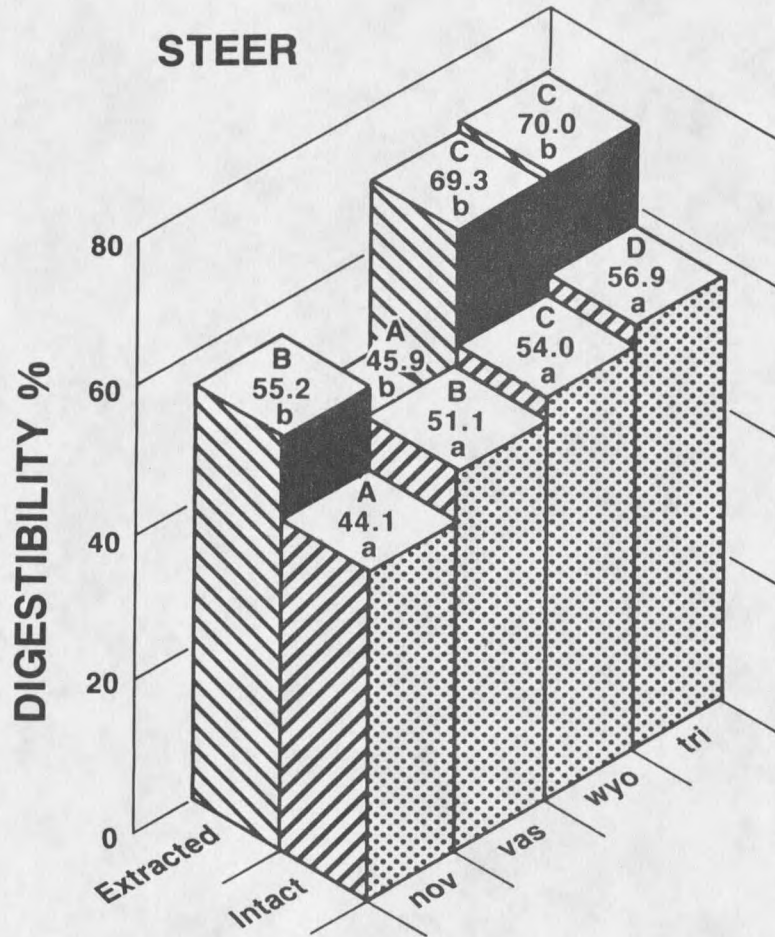


Figure 8. In vitro organic matter digestibility of 4 sagebrush taxa averaged for 3 dates (Jan. 1, Feb. 15, Apr. 1) using steer inoculum. Crude terpenoids were intact and extracted. Taxa: nov= black sagebrush; vas, wyo, tri= mountain, Wyoming and basin big sagebrushes, respectively. Extracted or intact values among taxa with unlike capital letters are significantly different ( $P < .05$ ). Values between extracted and intact of a given taxon with unlike small letters are significantly different ( $P < .05$ ).

sagebrushes were digested equally well by deer rumen inocula (Figure 6). For sheep the order of increasing digestibility was black sagebrush, Wyoming and basin big sagebrushes, each significantly different (Figure 7). For the steer, black sagebrush was significantly less digestible than Wyoming and basin big sagebrushes which were similar (Figure 8).

#### Digestibility During Winter

For intact sagebrush taxa there were consistent increases in IVOMD over collection dates in all trials (Figures 3, 4 and 5). For a given taxon, successive increases were not always significant. This was most apparent for the deer data which had the most variability (Figure 3). However, in all trials (Figures 3, 4 and 5) IVOMD on April 1 was significantly greater than on January 1 for any given intact sagebrush taxon, except basin big sagebrush - steer trial (Figure 5). When taxa IVOMD's were averaged for each collection date, there were significant increases in digestibility from January 1 to February 15 to April 1 in all trials (Figures 9, 10 and 11).

For extracted sagebrush taxa, trends in IVOMD over collection dates were inconsistent (Figures 3, 4 and 5). For a given taxon there were few significant changes in digestibility over time in all trials. When IVOMD for extracted taxa were averaged for each collection date, there was a significant increase in digestibility from January 1 to April 1 for the deer and sheep (Figures 9 and 10) but not for the steer (Figure 11).

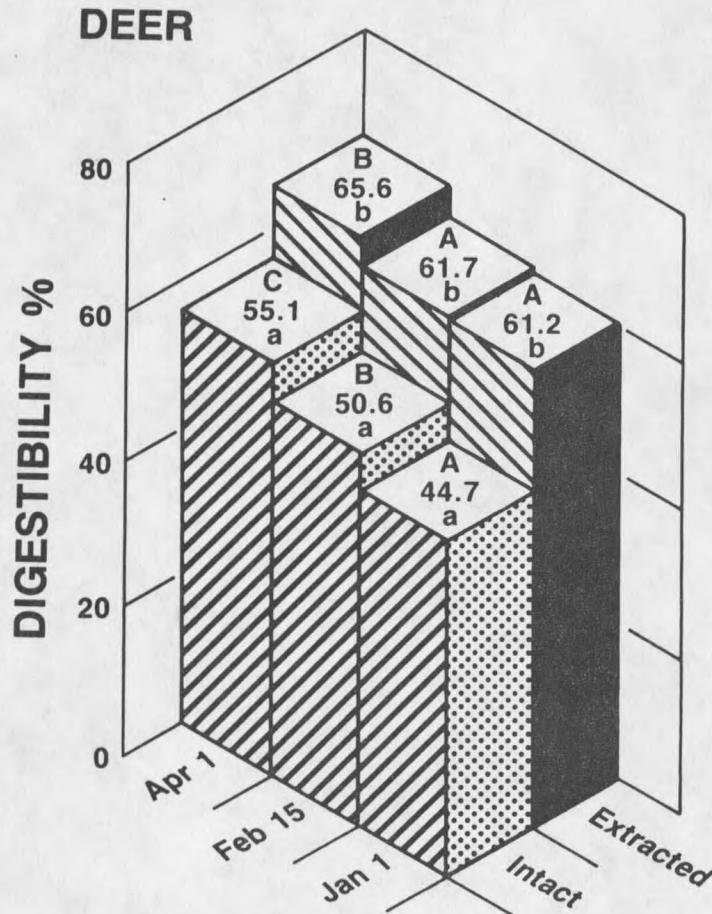


Figure 9. In vitro organic matter digestibility of sagebrush averaged for all taxa on 3 dates using deer inoculum. Crude terpenoids were intact and extracted. Taxa: black sagebrush; mountain, Wyoming and basin big sagebrushes. Extracted or intact values among dates with unlike capital letters are significantly different ( $P < .05$ ). Values between intact and extracted on a given date with unlike small letters are significantly different ( $P < .05$ ). Averages were computed using data for lightly and heavily grazed mountain and Wyoming big sagebrush (Appendix C, Tables 8 and 9).

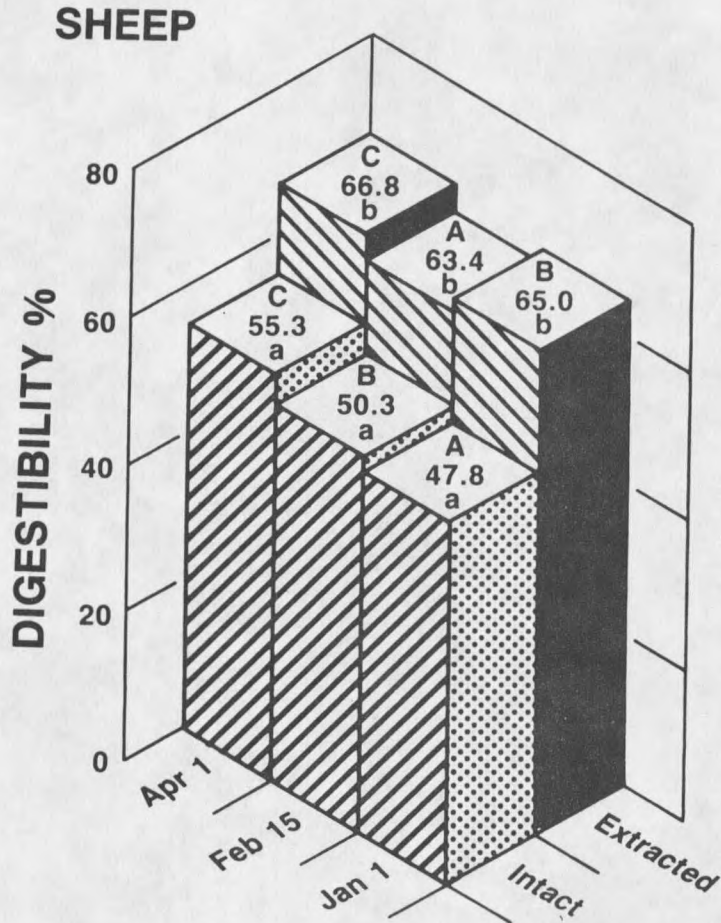


Figure 10. In vitro organic matter digestibility of sagebrush averaged for all taxa on 3 dates using sheep inoculum. Crude terpenoids were intact and extracted. Taxa: black sagebrush; mountain, Wyoming and basin big sagebrushes. Extracted or intact values among dates with unlike capital letters are significantly different ( $P < .05$ ). Values between intact and extracted on a given date with unlike small letters are significantly different ( $P < .05$ ). Averages were computed using data for lightly and heavily grazed mountain and Wyoming big sagebrush (Appendix C, Tables 8 and 9).

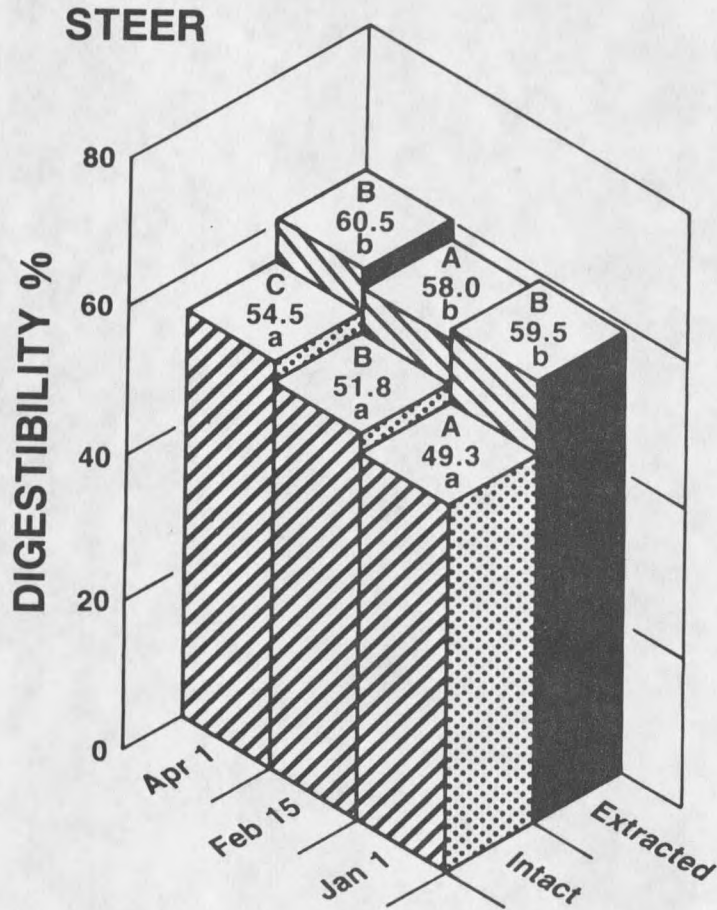


Figure 11. In vitro organic matter digestibility of sagebrush averaged for all taxa on 3 dates using steer inoculum. Crude terpenoids were intact and extracted. Taxa: black sagebrush; mountain, Wyoming and basin big sagebrushes. Extracted or intact values among dates with unlike capital letters are significantly different ( $P < .05$ ). Values between intact and extracted on a given date with unlike small letters are significantly different ( $P < .05$ ). Averages were computed using data for lightly and heavily grazed mountain and Wyoming big sagebrush (Appendix C, Tables 8 and 9).

### Digestibility of Intact versus Extracted Sagebrush

Extracted sagebrush (crude terpenoids removed) had significantly greater IVOMD than intact sagebrush (crude terpenoids not removed) in all trials (Figures 6-11). The only exception was the mountain big sagebrush in the steer trial (Figure 8) which was significantly less digestible than intact mountain big sagebrush. In vitro organic matter digestibility of extracted sagebrush averaged 13.3%, 14.9% and 11.4% higher than intact sagebrush for the deer, sheep and steer, respectively.

### Crude Terpenoid Concentration

No significant differences ( $P < .05$ ) in crude terpenoid concentration between lightly and heavily browsed mountain or Wyoming big sagebrush were detected by ANOV (Appendix D, Table 10). Therefore, concentration values for light and heavy categories were averaged in further data analyses. ANOV confirmed that sagebrush taxon and collection date were significant sources of variation (Figure 12). Taxon x date interactions were not detected.

### Intertaxon Concentration

Although all differences were not significant, crude terpenoid concentration among sagebrush taxa generally increased in the following order: mountain big sagebrush, black sagebrush, Wyoming and basin big sagebrushes (Figure 12). Statistical analysis of taxa averages over dates revealed that mountain big sagebrush contained the lowest level of crude terpenoids, black sagebrush and Wyoming big sagebrushes contained intermediate levels, followed by basin big sagebrush which

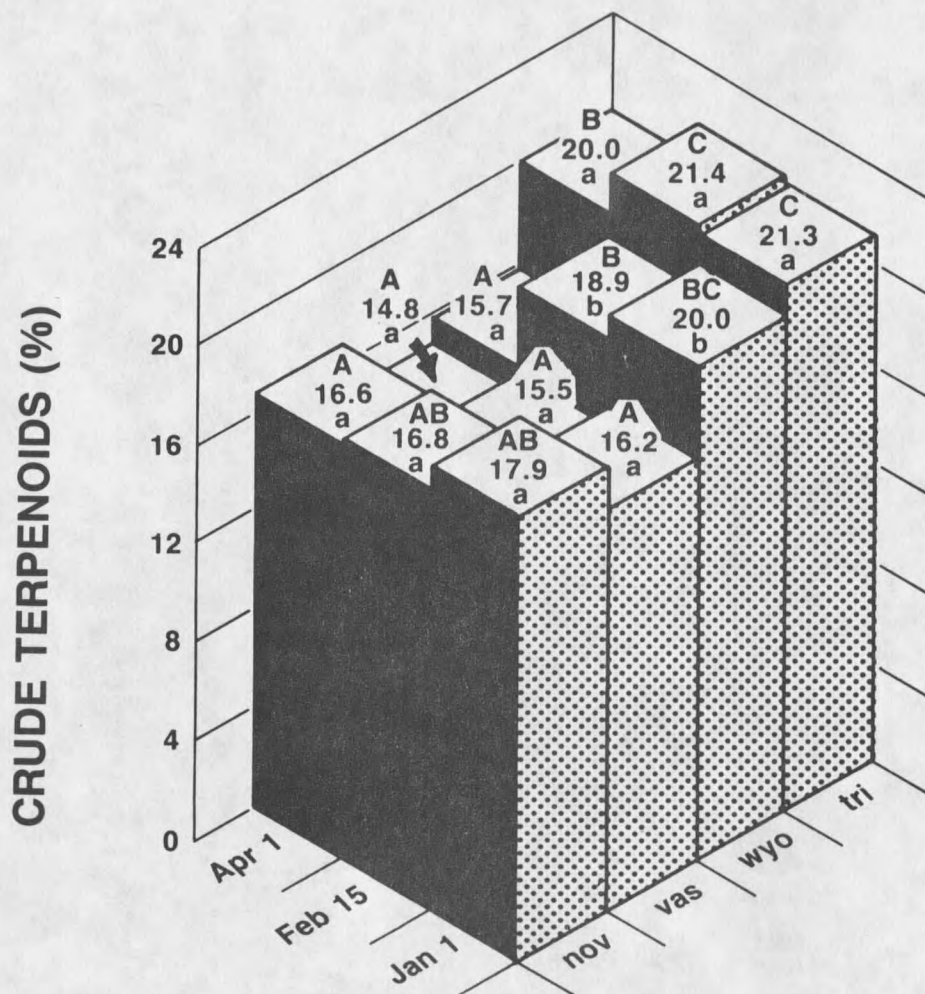


Figure 12. Crude terpenoid content (percent foliage dry weight) of 4 sagebrush taxa on 3 dates. Taxa: nov= black sagebrush; vas, wyo, tri= mountain, Wyoming and basin big sagebrushes, respectively. Values among taxa on a given date with unlike capital letters are significantly different ( $P < .05$ ). Values among dates for a given taxon with unlike small letters are significantly different ( $P < .05$ ).

contained the highest levels of crude terpenoids (Figure 13).

#### Concentration During Winter

For each sagebrush taxon, crude terpenoid concentration generally decreased over time; however, few significant differences were apparent (Figure 12). When taxa concentrations were averaged for each collection date the average crude terpenoid concentration on April 1 was significantly less than the average concentrations on February 15 and January 1 which were similar (Figure 14).

#### Correlation Between Crude Terpenoid Concentration and Digestibility

The relationship between crude terpenoid concentration and IVOMD is graphically illustrated for each digestion trial in Figures 15, 16 and 17. There was a general negative relationship between crude terpenoid concentration and digestibility within each sagebrush taxon in all trials. Within each taxon, as concentration decreased, digestibility increased. However, this relationship did not apply between sagebrush taxa. Basin big sagebrush had the highest crude terpenoid concentration, but also had the highest digestibility in all trials. Mountain big sagebrush, which had the lowest crude terpenoid concentration was intermediate in digestibility between black sagebrush and Wyoming big sagebrush.

Analysis of covariance using crude terpenoid concentration as a covariant revealed no significant effect of crude terpenoid concentration on sagebrush IVOMD when sources of variation due to sagebrush taxa and collection dates were included in ANCOV tables. However, when source of variation due to collection date was excluded from ANCOV, there was a significant negative relationship ( $P < .01$ ) between crude

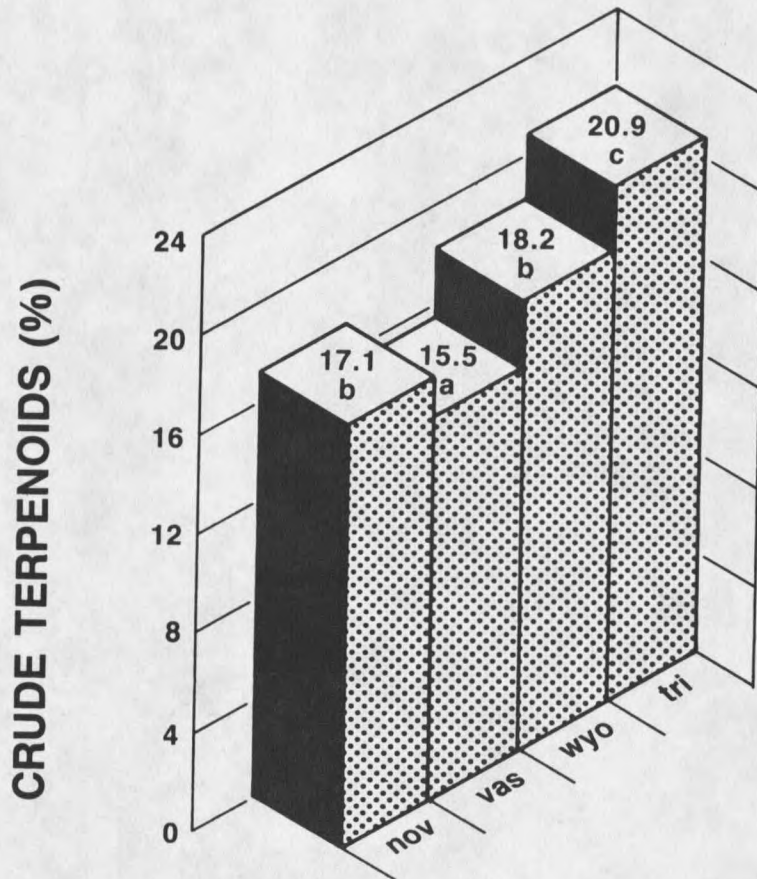


Figure 13. Crude terpenoid content (percent foliage dry weight) of 4 sagebrush taxa averaged for 3 dates (Jan. 1, Feb. 15, Apr. 1). Taxa: nov= black sagebrush; vas, wyo, tri= mountain, Wyoming and basin big sagebrushes, respectively. Values with unlike letters are significantly different ( $P < .05$ ).

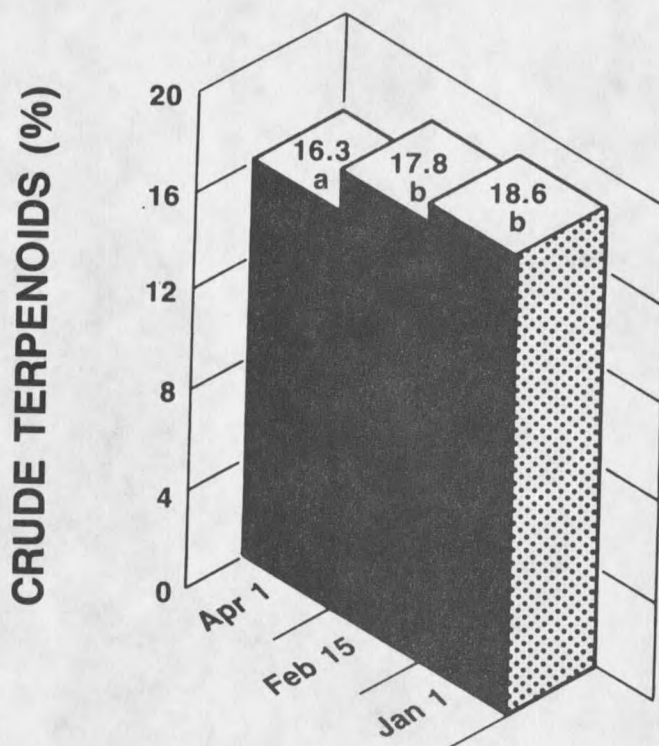


Figure 14. Crude terpenoid content (percent foliage dry weight) of sagebrush averaged for all taxa on 3 dates. Taxa: black sagebrush; mountain, Wyoming and basin big sagebrushes. Values with unlike letters are significantly different ( $P < .05$ ). Averages were computed using data for lightly and heavily grazed mountain and Wyoming big sagebrush (Appendix D, Table 10).

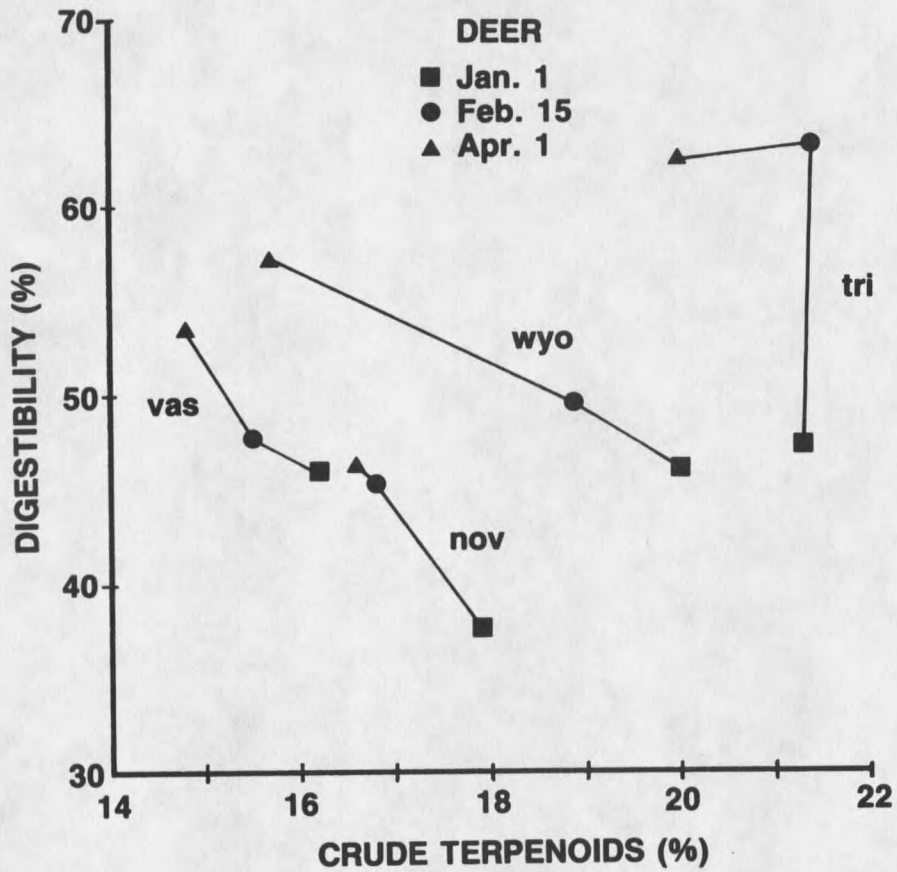


Figure 15. In vitro organic matter digestibility of 4 sagebrush taxa versus crude terpenoid content (percent foliage dry weight) on 3 dates. Rumen inocula donor was a deer. Taxa: nov= black sagebrush; vas, wyo, tri= mountain, Wyoming and basin big sagebrushes, respectively.

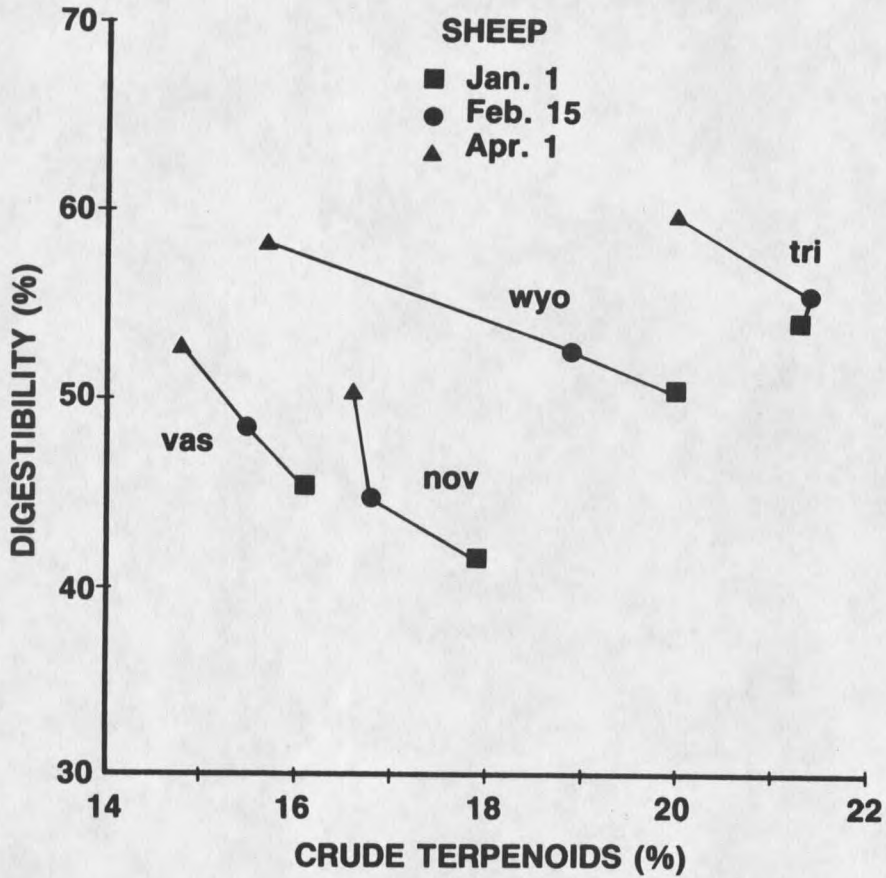


Figure 16. In vitro organic matter digestibility of 4 sagebrush taxa versus crude terpenoid content (percent foliage dry weight) on 3 dates. Rumen inocula donor was a sheep. Taxa: nov= black sagebrush; vas, wyo, tri= mountain, Wyoming and basin big sagebrushes, respectively.

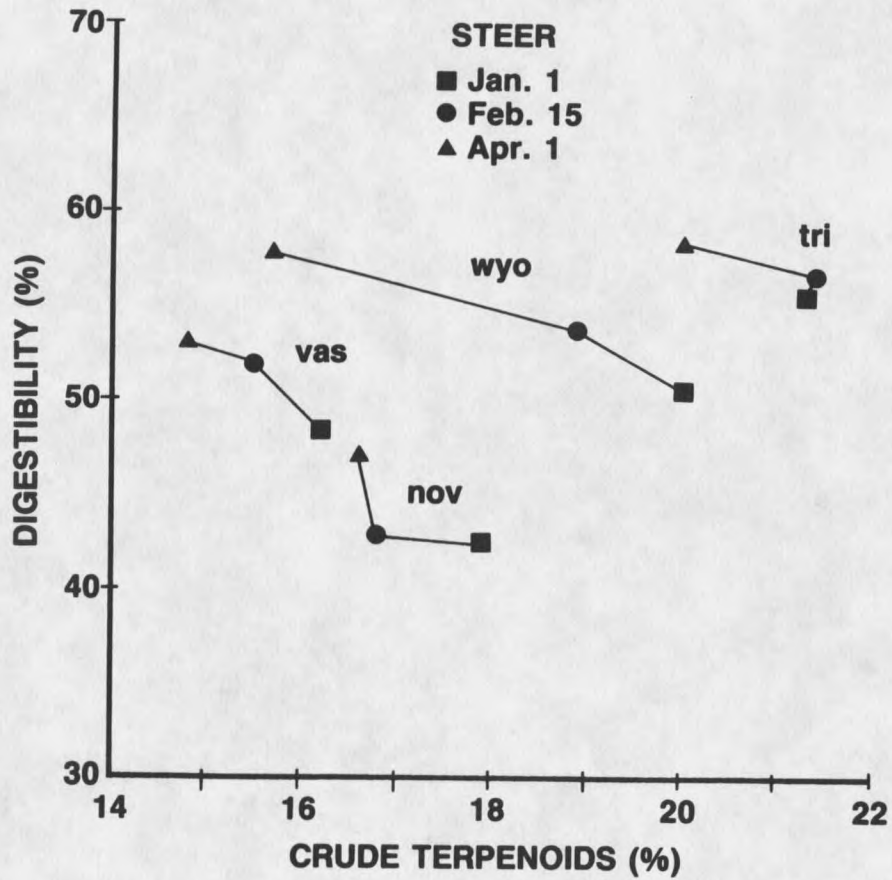


Figure 17. In vitro organic matter digestibility of 4 sagebrush taxa versus crude terpenoid content (percent foliage dry weight) on 3 dates. Rumen inocula donor was a steer. Taxa: nov= black sagebrush; vas, wyo, tri= mountain, Wyoming and basin big sagebrushes, respectively.

terpenoid concentration and digestibility in all trials. Correlation coefficients for the deer, sheep and steer trials were -0.72, -0.77 and -0.83, respectively. The small sample size did not permit calculation of meaningful regression equations within taxa.

#### Relative Utilization of Sagebrush Taxa

Form class, indicating degree of hedging from past use, was assigned to a total of 1569 sagebrush plants. The total number of black sagebrush, mountain, Wyoming and basin big sagebrush plants observed was 460, 439, 354 and 316, respectively. Distribution among form classes is shown in Table 6. Percentage frequency occurring in each form class is shown in Table 7. Chi-square analysis for each taxon disproved the null hypothesis that the probability of occurrence in any particular form class is one out of three. Wyoming big sagebrush had the highest percentage (89%) of plants in the heavy form class, and basin big sagebrush had the highest percentage (82%) of plants in the light form class (Table 7). Approximately 98% of the observed mountain big sagebrush plants were moderately to heavily grazed, and approximately 87% of the black sagebrush plants were lightly to moderately grazed (Table 7).

Table 6. Distribution of plants observed for degree of hedging from past use among form classes.

Taxon	Form Class			Plants Observed
	light	Moderate	Heavy	
<u>A. nova</u>	94	308	58	460
<u>A. t. ssp. vas</u>	23	198	218	439
<u>A. t. ssp. wyo</u>	0	39	315	354
<u>A. t. ssp. tri</u>	258	51	7	316
Total observed				1569

Table 7. Percentage frequency distribution of plants observed for degree of hedging from past use among form classes.

Taxon	Form Class		
	light	Moderate	Heavy
<u>A. nova</u>	20.4	67.0	12.6
<u>A. t. ssp. vas</u>	5.2	45.1	49.7
<u>A. t. ssp. wyo</u>	0	11.0	89.0
<u>A. t. ssp. tri</u>	81.7	16.1	2.2

## DISCUSSION

The most pronounced observation supporting the implication that crude terpenoid compounds influence the digestibility of sagebrush was the significantly higher IVOMD of the extracted over the intact sagebrush (Figures 3-11). After extraction of crude terpenoids, the average IVOMD for all taxa, collection dates and inocula sources increased by 13.2%. Two basic hypotheses follow. On one hand, the crude terpenoid compounds might independently or synergistically play an active role in microbial inhibition. On the other hand, the crude terpenoids may play a passive role by merely being inert substances that are impervious to microbial digestion. Either one, or both of these hypotheses might be partially responsible for the observed effects. Each will be discussed, beginning with the possible passive, inert role of crude terpenoids.

The crude terpenoid compounds may be divided into 2 general fractions based on their volatility. The monoterpenes, which are highly volatile, may comprise as much as 9% of the foliage dry weight; the sesquiterpene lactones, phenolics and cuticular compounds, collectively are of low volatility and may comprise approximately 6-16% of the foliage dry weight (Table 1). Recognition of this basic difference in volatility is critical to the following discussions concerning the influence that these compounds might have on *in vitro* digestion.

In order to calculate either IVDM or IVOMD, the dry or organic matter content of both forage and undigested residue must be

determined. In making these determinations, the forage and residue are oven dried at 100-105°C. Welch and Pederson (1981) demonstrated that oven drying sagebrush at 100°C resulted in a total loss of monoterpenes. Consequently, monoterpenes, like water, would not be represented in the quantity of dry or organic matter in either the forage or the residue and therefore, would not enter into the calculation of IVDM or IVOMD.

Unlike the monoterpenes, the non-volatile crude terpenoids (sesquiterpene lactones, phenolics and cuticular compounds) remain in oven dried materials because of their high boiling and melting points. Consequently, these compounds would be represented in the quantity of forage dry or organic matter placed in fermentation tubes. Since they possess a variety of biological activities (Table 1) it seems likely that the non-volatile crude terpenoids are not digested by rumen microorganisms. Some of these compounds are also resistant to the hot water washes employed during the filtration of the undigested forage residue. Kelsey et al. (1982) reported that fall sagebrush foliage which had been subjected to steam distillation for 2 hours (a process much more rigorous than washing with hot water) contained 66% of the ether soluble crude fat (much of which is composed of non-volatile crude terpenoids) present in fresh material. Therefore, the quantity of non-volatile crude terpenoids contained in the undigested forage residue after oven drying would be subtracted from the quantity of forage dry or organic matter placed in fermentation tubes, thus lowering in vitro digestibility of intact sagebrush.

Conversely, it seems likely that removal of the non-volatile

crude terpenoids would in effect concentrate the more digestible forage fractions, thus increasing the *in vitro* digestibility of extracted sagebrush. Kelsey et al. (1982) found that chloroform extraction of the crude terpenoids from mountain big sagebrush concentrated total nonstructural carbohydrates (TNC) and only slightly reduced crude protein levels in the foliage. The TNC are nearly 100% digestible (Van Soest 1967).

This research did not quantify the volatile and non-volatile fractions of the crude terpenoids in either sagebrush or *in vitro* residues. Furthermore, the nutrient composition of intact and extracted sagebrush was not determined. Such measures would be needed to test the above hypothesis concerning the passive role of crude terpenoids in *in vitro* digestion.

Recognition of the two basic volatility classes within the crude terpenoids is also essential to the discussion of the possible active involvement of these compounds in microbial inhibition. Welch and Pederson (1981) reported that the temperature at which *in vitro* digestion tubes are incubated (38.5° C) was sufficient to volatilize monoterpenoids and therefore, they do not significantly interact with rumen microorganisms. However, this temperature is not high enough to drive off the relatively non-volatile sesquiterpene lactones and phenolics (coumarins and flavnoids). Substantial quantities of these compounds are present in sagebrush foliage and many possess a wide range of biological activities (Table 1). However, as discussed in the literature review of this thesis, past studies have overlooked the influence of these compounds on rumen microbial activity. Based on

these factors, it seems possible that sesquiterpene lactones and phenolics might independently or together cause a reduction in microbial digestion of sagebrush tissues.

The preceding hypotheses can be broadened to explain the variability in IVOMD observed among sagebrush taxa and collection dates. Hence, the following will evaluate the influence of observed differences in crude terpenoid concentration on IVOMD. The effects of crude terpenoid and nutrient composition will also be discussed.

Analysis of covariance detected a significant negative relationship between crude terpenoid concentration and IVOMD of intact samples when taxa were combined and the variation due to collection date was not considered ( $r = -.72, -.77$  and  $-.83$  for the deer, sheep and steer trials, respectively). Figures 15, 16 and 17 revealed that the correlation was based on within taxon differences. This relationship can be explained by either hypotheses concerning the passive or active involvement of crude terpenoids in microbial digestion. For example, as the amount of non-volatile, non-digestible crude terpenoids increase within a particular taxon, a smaller percentage of the foliage organic matter would be available for microbial digestion and IVOMD would decrease proportionally. Similarly, if the non-volatile crude terpenoids decrease microbial activity, it seems logical to assume that as concentration increases within a particular taxon, microbial inhibition would increase, thus decreasing IVOMD.

Since crude terpenoid concentration generally decreased over time (Figures 12, 14), ANCOV showed no significant relationship between crude terpenoid concentration and IVOMD when collection date was

included as a source of variation. This is demonstrated graphically in Figures 15, 16 and 17 by ignoring taxa and date groupings of the data points; overall, as crude terpenoid concentration increases, there is no meaningful effect on digestibility. Taxa having successively higher crude terpenoid concentrations were not successively less digestible.

The composition of the secondary metabolic products of sagebrush are under genetic control (Shafizadeh and Melnikoff 1970, Kelsey et al. 1976, Welch and McArthur 1981, Kelsey et al. 1982). Potential compositional differences among the crude terpenoid extracts of the various taxa might account for the inconsistent effect of crude terpenoid concentration on IVOMD observed among taxa.

For example, basin big sagebrush had the highest crude terpenoid concentration and highest IVOMD (Figures 15, 16 and 17). It also may have had the highest concentration of monoterpenes and lowest concentration of non-volatile crude terpenoid compounds that are resistant to filtration of the undigested residue. Consequently, the high concentration of monoterpenes would not affect IVOMD since they are driven off by heat, and the low concentration of non-volatile compounds left in the undigested residue would cause little reduction in IVOMD. Kelsey et al. (1982) reported that the winter levels of monoterpenes in basin big sagebrush were higher than the levels found in mountain and Wyoming big sagebrush. In addition, the non-volatile crude terpenoid compounds in basin big sagebrush might have little effect on microbial activity.

Conversely, mountain big sagebrush, which had the lowest crude

terpenoid concentration and second lowest IVOMD (Figures 15, 16 and 17), may have had a relatively low concentration of monoterpenes and a relatively high concentration of non-volatile compounds resistant to the residue filtration process. Therefore, its IVOMD would have been substantially reduced by a high concentration of non-volatile compounds remaining in the forage residue. Secondly, the non-volatile crude terpenoid compounds in mountain big sagebrush might have substantially inhibited rumen microorganisms.

Nutrient fractions that influence sagebrush digestibility such as TNC, crude protein and crude fat are under genetic and phenological control (Welch and McArthur 1979, Kelsey et al. 1982). If the observed variation in IVOMD of intact sagebrush was caused by nutrient fluctuations, it follows that trends in IVOMD of intact and extracted sagebrush would be similar. However, the trends in IVOMD that were observed for the intact samples were not apparent in the IVOMD's of the extracted samples (Figures 3-11). For example, in the steer trial, increasing order of digestibility among intact taxa was black sagebrush, mountain, Wyoming and basin big sagebrushes (Figure 8); increasing order of digestibility among collection dates was January 1, February 15 and April 1 (Figure 11). All differences between taxa and dates were significant. However, most of the trends and differences in IVOMD among taxa and dates of extracted sagebrush in the steer trial were either not the same, and/or not significant when compared to intact sagebrush (Figures 8 and 11). This indicates that crude terpenoids do influence the IVOMD's of intact sagebrush. The trends and significant differences in IVOMD among taxa and dates of extracted sagebrush

characterize the influence that nutrient fractions, (i.e. TNC and cell wall constituents) have on digestibility.

The assignment of form class to 1569 sagebrush plants revealed degree of hedging from combined deer and elk utilization. Since the four taxa occurred within 0.5 to 1.0 ha, animals could select among the taxa in cafeteria fashion. Form class assessment was interpreted as an indication of the relative big game preference for the taxa. Based on the percentage frequency distribution of the taxa among form classes (Table 7), order of increasing preference appeared to be basin big sagebrush, black sagebrush, mountain big sagebrush, and Wyoming big sagebrush. These results are in general agreement with various reports that rated Wyoming and mountain big sagebrush more preferred than basin big sagebrush by livestock and game (Hanks et al. 1973, Stevens and McArthur 1974, McArthur et al. 1979 and Beetle and Johnson 1982); and mountain big sagebrush more preferred by deer and sheep than basin big sagebrush (Sheehy 1975, Scholl 1977, Welch et al. 1981). The black sagebrush observed in this study may have been the less preferred dark green ecotype reported by Brunner (1972), Stevens and McArthur (1974) and McArthur et al. (1979) who ranked it less preferred than Wyoming and mountain big sagebrush by livestock and game.

Oh et al. (1967), Longhurst et al. (1968), Radwan (1972), Dietz and Nagy (1976) and Schwartz et al. (1980b) reported that deer can recognize the harmful effects of essential oils on rumen microbes and select against those species or individual plants containing essential oils with the greatest antimicrobial effects. This line of reasoning

suggests that the most preferred plants are also the most digestible (Longhurst et al. 1968). On the contrary, this study demonstrated that IVOMD of lightly and heavily grazed mountain and Wyoming big sagebrush plants of the same taxon and growing side by side were similar (Appendix C, Table 8). This indicates that the differential grazing preference exhibited for individual plants of a particular taxon was not related to digestibility. Furthermore, there was no consistent positive correlation between grazing preferences displayed among taxa and digestibility. For example, basin big sagebrush appeared to be the least preferred taxon, yet had the highest average IVOMD (Figures 6-8).

Similarly, preference did not appear to be related to crude terpenoid concentration either within or among taxa. ANOV revealed that crude terpenoid concentrations of lightly and heavily grazed plants of the same taxon and growing side by side were not significantly different (Appendix D, Table 10). Wyoming big sagebrush appeared to be the most preferred taxon, yet its average crude terpenoid concentration was not significantly different from black sagebrush which was not highly preferred (Figure 13).

Animal preferences for the various sagebrush taxa are probably based on differences in smell or taste of crude terpenoid compounds rather than their antimicrobial properties (Kelsey et al. 1983, Narjisse 1981). Narjisse (1981) found that sheep discriminated against monoterpene odor, while goats discriminated against the taste. The crude terpenoids contain a wide variety of compounds, each having a distinct sensory perception (Table 1). It seems probable that

differences in the composition of these compounds, rather than total concentration, may cause differences in smell or taste that stimulate a selective grazing response.

## SUMMARY AND CONCLUSIONS

The purpose of this study was to evaluate the relative forage quality of black sagebrush and mountain, Wyoming and basin big sagebrushes as reflected in in vitro organic matter digestibility (IVOMD), crude terpenoid concentration and animal grazing preference. Current years growth was collected from the sagebrush taxa on January 1, February 15 and April 1, 1981. Crude terpenoids were extracted and quantified. The IVOMD of extracted and intact (crude terpenoids not extracted) sagebrush was determined using rumen fluid inocula from a mule deer, sheep and steer. A total of 1569 sagebrush plants representing the 4 taxa and growing in so-called "cafeterias" were examined for degree of hedging from combined deer and elk utilization. Utilization assessment gave an indication of the relative big game preference for the taxa. Based on the experimental observations the following conclusions are presented:

1. Order of increasing IVOMD among intact sagebrush taxa was black sagebrush, mountain, Wyoming and basin big sagebrushes, each significantly different. There were fewer significant differences in IVOMD among extracted sagebrush taxa; mountain big sagebrush was consistently less digestible than black sagebrush which was generally less digestible than Wyoming and basin big sagebrushes. The IVOMD of intact sagebrush generally increased over all collection dates, whereas the IVOMD of extracted sagebrush generally was unchanged. General trends in IVOMD among sagebrush taxa and collection dates and

between intact and extracted material were nearly the same for all inocula sources.

2. Utilization assessment indicated that Wyoming big sagebrush was the most preferred taxon. Mountain big sagebrush, black sagebrush and basin big sagebrush, respectively were progressively less preferred.

3. Mountain big sagebrush contained the lowest level of crude terpenoids followed by black sagebrush and Wyoming big sagebrush, which contained intermediate levels, and basin big sagebrush which contained the highest level. Crude terpenoid concentrations for all sagebrush taxa were greater on January 1 and February 15 than on April 1.

4. Non-volatile crude terpenoid compounds decreased the IVOMD of sagebrush through microbial inhibition and/or their resistance to digestion. Within a particular taxon, IVOMD decreased as crude terpenoid concentration increased. Crude terpenoid composition might be responsible for the variability in IVOMD observed among taxa. Even though non-volatile crude terpenoids decreased IVOMD, sagebrush digestibility remained high relative to other winter range forages.

5. The variation in IVOMD and crude terpenoid concentration among the 4 sagebrush taxa was not related to utilization differences. In addition, lightly and heavily grazed plants of either mountain or Wyoming big sagebrush were digested equally well and contained similar crude terpenoid concentrations. Therefore, apparent animal grazing preference was not influenced by either digestibility or crude

terpenoid concentration. However, crude terpenoid composition may influence forage selection through the stimulation of smell and/or taste.

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## LITERATURE CITED

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**APPENDICES**

APPENDIX A  
STATISTICAL MODEL

## Statistical Model

Mathematically the model may be written:

$$\bar{Y}_{ijkl} = u + T_i + D_j + (TD)_{ij} + M_{ijk} + P_{ijl}$$

where  $i = 1 \dots 6$ ,  $j = 1 \dots 3$ ,  $k = 1 \dots 4$  (intact) or  $1 \dots 2$  (extracted and crude terpenoid),  $l = 1 \dots 20$ .

Any observed value is the sum of six parts: (i) an overall mean, (ii) a deviation of the  $i$ th taxon category, (iii) a deviation of the  $j$ th collection date, (iv) the two-factor interaction effects, (v) the deviation of the  $k$ th measurement, and (vi) the deviation associated with the  $l$ th plant.

APPENDIX B  
ERROR MEAN SQUARE TERMS

## Error Mean Square Terms

Error mean square terms were constructed separately for each digestion trial (mule deer, domestic sheep, steer) and crude terpenoid data. Equations, based on the statistical model, were formulated by Dr. R. E. Lund, associate professor of statistics, Montana State University.

For intact data an estimate of variance of  $\bar{Y}_{ijk}$  was based on plant to plant variation ( $\sigma_p^2$ ) and error associated with each measurement ( $\sigma_m^2$ ) as follows:

$$\text{Vâr} (\bar{Y}_{ijk}) = \frac{\hat{\sigma}_p^2}{5} + \hat{\sigma}_m^2 = \text{pooled } s_{int}^2$$

Variance attributed to plant variation ( $\hat{\sigma}_p^2$ ) was divided by 5 since each IVOMD measurement was for five plants.

For extracted and crude terpenoid data an estimate of variance of  $\bar{Y}_{ijk}$  was based on measurement error alone ( $\hat{\sigma}_m^2$ ) since each determination was for all twenty plants.

$$\text{Vâr} (\bar{Y}_{ijk}) = \hat{\sigma}_m^2 = \text{pooled } s_{ext}^2$$

The pooled  $s_{ext}^2$  was substituted for  $\hat{\sigma}_m^2$  in the intact equation and solved for  $\hat{\sigma}_p^2$  as follows:

$$\frac{\hat{\sigma}_p^2}{5} + \text{pooled } s_{ext}^2 = \text{pooled } s_{int}^2$$

$$\frac{\hat{\sigma}_p^2}{5} = \text{pooled } s_{int}^2 - \text{pooled } s_{ext}^2$$

$$\hat{\sigma}_p^2 = \frac{\text{pooled } s_{int}^2 - \text{pooled } s_{ext}^2}{5}$$

By utilizing the value calculated for  $\hat{\sigma}_p^2$  and  $\hat{\sigma}_m^2$ , estimates of the variance of  $\bar{Y}_{ij}$ , the error mean square, were constructed.

For intact data:

$$\text{EMS} = \text{Vâr} (\bar{Y}_{ij}) = \frac{\hat{\sigma}_p^2 + \hat{\sigma}_m^2}{\frac{5}{4}}$$

The denominator of the term on the right is 4 since the IVOMD mean for the ith sagebrush category and the jth collection date was comprised of 4 measurements. The intact error mean squares could also be constructed as

$$\text{Vâr} (\bar{Y}_{ij}) = \frac{\text{pooled } s_{\text{int}}^2}{4}$$

For extracted and crude terpenoid data:

$$\text{EMS} = \text{Vâr} (\bar{Y}_{ij}) = \frac{\hat{\sigma}_p^2}{20} + \frac{\hat{\sigma}_m^2}{2}$$

The estimation of plant to plant variation ( $\hat{\sigma}_p^2$ ) was added to measurement error ( $\hat{\sigma}_m^2$ ). Respective denominators were 20 and 2 since each of two measurements were for twenty plants.

The crude terpenoid data supplied an estimate of  $\hat{\sigma}_m^2$  only. Therefore, the  $\hat{\sigma}_p^2$  for crude terpenoid was supplied by taking the average ratio of  $\hat{\sigma}_p^2$  to  $\hat{\sigma}_m^2$  from the IVOMD data and multiplying by the  $\hat{\sigma}_m^2$  for crude terpenoids as follows:

$$\hat{\sigma}_p^2 \text{ crude terpenoids} = \frac{\hat{\sigma}_p^2}{\hat{\sigma}_m^2} \text{ ave. for IVOMD's} \times \hat{\sigma}_m^2 \text{ crude terpenoids.}$$

APPENDIX C

IVOMD OF LIGHTLY AND HEAVILY GRAZED  
MOUNTAIN AND WYOMING BIG SAGEBRUSH

Table 8. IVOMD of lightly and heavily grazed mountain and Wyoming big sagebrush using deer, sheep and steer rumen inocula - Intact samples.

Sagebrush category <sup>1</sup>	Collection Date		
	Jan. 1	Feb. 15	Apr. 1
		<u>Mule Deer</u>	
vas-light	43.2	45.9	53.2
vas-heavy	48.5	50.0	53.7
wyo-light	46.2	50.1	62.0
wyo-heavy	45.8	48.9	52.7
		<u>Sheep</u>	
vas-light	46.4	48.3	52.2
vas-heavy	44.4	48.7	53.3
wyo-light	50.3	52.4	56.9
wyo-heavy	50.3	52.5	59.3
		<u>Steer</u>	
vas-light	49.3	51.6	53.0
vas-heavy	47.3	52.5	53.0
wyo-light	50.7	52.9	57.7
wyo-heavy	50.3	54.4	57.9

<sup>1</sup>vas-light, vas-heavy = mountain big sagebrush - lightly and heavily browsed, respectively; wyo-light, wyo-heavy = Wyoming big sagebrush - lightly and heavily browsed, respectively.

Table 9. IVOMD of lightly and heavily grazed mountain and Wyoming big sagebrush using deer, sheep and steer rumen inocula -- Extracted samples.

Sagebrush category <sup>1</sup>	Collection Date		
	Jan. 1	Feb. 15	Apr. 1
		<u>Mule Deer</u>	
vas-light	58.6	59.3	61.0
vas-heavy	60.9	53.5	56.1
wyo-light	66.1	56.2	67.4
wyo-heavy	61.7	69.5	67.5
		<u>Sheep</u>	
vas-light	57.7	54.7	59.0
vas-heavy	57.4	52.9	57.2
wyo-light	68.9	67.6	71.2
wyo-heavy	69.3	69.5	73.5
		<u>Steer</u>	
vas-light	49.3	44.0	48.1
vas-heavy	46.4	43.5	46.8
wyo-light	69.7	68.1	68.9
wyo-heavy	69.0	69.3	71.7

<sup>1</sup> vas-light, vas-heavy = mountain big sagebrush - lightly and heavily browsed, respectively; wyo-light, wyo-heavy = Wyoming big sagebrush - lightly and heavily browsed, respectively.

APPENDIX D

CRUDE TERPENOID CONCENTRATION OF LIGHTLY AND  
HEAVILY GRAZED MOUNTAIN AND WYOMING BIG SAGEBRUSH

Table 10. Crude terpenoid concentration of lightly and heavily grazed mountain and Wyoming big sagebrush.

Sage brush category <sup>1</sup>	Collection Date		
	Jan. 1	Feb. 15	Apr. 1
vas-light	17.0	15.8	14.9
vas-heavy	15.4	15.2	14.8
wyo-light	20.3	18.6	14.7
wyo-heavy	19.7	19.0	16.8

<sup>1</sup> vas-light, vas-heavy = mountain big sagebrush - lightly and heavily browsed, respectively; wyo-light, wyo-heavy = Wyoming big sagebrush - lightly and heavily browsed, respectively.

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