

AN ABSTRACT OF THE THESIS OF

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Title: Nutritional Value of Several Opuntia Species

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Little is known about cactus nutrients, their proportions in different species and the variability of these proportions with season. In the scant literature available on Opuntia species, no systematic survey has been published to document and compare the nutritional qualities of different species. Also, no studies have been done to determine the seasonal variability of these nutrients. Past work on cactus has been mostly concentrated on Opuntia ficus-indica.

This study was designed to determine and compare major pad nutrient content in five Opuntia species, and assess the seasonal variability in one of them: Opuntia fragilis (from John Day Fossil Beds, Central Oregon). The other species included in the analyses were: O. engelmannii, O. polyacantha, O. filipendula and O. versicolor, and were collected from different regions in the Southwestern United States.

Differences in nutrient content were detected between the species included in the analyses, but no single or group of species was consistently low or high in all the components analyzed. O. engelmannii was the lowest in crude fiber components: 31.18% cell wall constituents, 7.9% cellulose and 2.89% lignin. Consequently, this species had the highest In vivo crude protein (51.80%), In vivo and In vitro dry matter digestibility (57.22% and 59.22%, respectively). The energy measurements indicated that, this species, O. engelmannii had the highest digestible energy (2098.54 kcal/kg). Conversely, O. versicolor, the unique cylindropuntia included in the study, had the highest crude fiber content: 39.85% as Neutral Detergent Fiber residue, 13.73% as cellulose and 3.86% as lignin (second highest). With regard to dry matter digestibility, this species was among the group of lowest rates: 48.30% and 53.62 % respectively as In vivo and In vitro levels. This species, O. versicolor, had the highest crude protein content (6.83%), and the second highest in digestible energy (1927.15 kcal/kg) but it was the lowest in phosphorus concentration (0.08%). O. polyacantha had the highest lignin content (4.79%) and the lowest In vivo dry matter and crude protein degradability rates: 44.61% and 36.61%, respectively. The mineral analyses resulted in a highest calcium level for O. filipendula (6.83%) and a

highest phosphorus content in the case of O. fragilis (0.29%).

With regard to the seasonal variability, the analyses indicated a general trend of increase in fiber and calcium content from spring to summer. Nearly, these nutrients maintained constant proportions from summer to fall. This was primarily due to the fact that spring growth has reached maturity during this period. A slight decline of fiber content occurred from fall to winter: More soluble components have been synthesized during this period. Phosphorus content was not significantly different between seasons. Spring samples had highest crude protein (3.73%) and digestible energy (1922.33 kcal/kg) content. This was mainly due to rapid growth of the plant, considerable quantity of young pads was included in the spring collection.

The seasonal variability was not highly significant. Moreover, content differences between seasons were not very high and a large part of these statistical variability could be inherent to the sampling procedure used. From a nutritional stand point, these small differences should not be taken into consideration in feeding O. fragilis to livestock through the year.

All the five Opuntia species analyzed resulted in lower crude fiber content and higher dry matter and crude protein digestibility than grass hay samples (Agropyron

cristatum and Bromus spp.) analyzed under the same conditions. When compared to alfalfa (Medicago sativa), cactus pads appeared to be intermediate in forage quality. All the cactus samples had higher calcium levels than the samples reference. In addition, O. engelmannii had dry matter and crude protein digestibility rates close to those of alfalfa samples. Its digestible energy was only 20% lower than that of alfalfa. From this it could be concluded that the Opuntia species analyzed can fit in livestock rations with attention given to their low phosphorus and protein content. These Opuntias could be fed during any season but are needed, the most, as a late summer and fall supplement and during droughts when other forages are scarce or expensive to feed.

Nutritional Value of Several
Opuntia Species

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DEDICATION

This thesis is dedicated, with all my love and respect to my parents, AMMAR BEN-THLIJA and MABROUKA BENT MOHAMMEM THLIJA as a recognition for their support and precious sacrifices. Also this thesis is dedicated with love and respect to my wife, HAYET DJELASSI BEN-THLIJA and my beloved daughters IBTISAM and SALWA for their long patience and helpful sacrifices.

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NUTRITIONAL VALUE OF SEVERAL

OPUNTIA SPECIES

INTRODUCTION

Farmers in central and southern Tunisia as well as in the southwestern United States and other places in the world are frequently confronted with periods of drought which endanger the well-being, if not the actual existence, of their livestock. Under such circumstances these farmers are forced to, either buy feeds, or move their animals to other regions where grazing is available. Both alternatives are expensive and not permanent. Another alternative is that the rancher sells a certain number (usually significant) of his animals to save the stock from starvation. This approach to solving the problem is disadvantageous in several ways, particularly with low market price usually dictated by drought conditions, and the difficulties associated with rebuilding the herd when the situation becomes better.

It is possible to find other durable and more efficient remedies to such periods of forage shortage by using drought-resistant plants as a feed source. Included in this category of plants are cacti. Cacti endure severe climatic conditions and provide a valuable forage that could be used as a supplement or even fed alone to maintain livestock over relatively long periods.

PHYLOGENY, DISTRIBUTION AND USE OF CACTUS

Cactus is a common name for tree-like and shrubby plants of the Cactaceae family (genus: Opuntia). From the literature, it appears that very little is known about the phylogeny of this family. According to Lofgreen (Teles, 1978) the family Cactaceae is found isolated in the taxonomic system. This is probably due to any of several simultaneous or isolated causes including; the cactaceae do not appear in the fossil flora because of anatomical structures that prevent their preservation, or it could be that this family is not old enough for paleontological study.

Most of the Cactaceae family representatives known today can be traced back to the American continent. Teles (1978) indicated that cactus species undoubtedly originated from the tropical and subtropical areas of the New World. The author thinks that, because of the great number of species found in Mexico, that cacti had spread from that region to the other parts of the world.

From its site of origin, cacti have spread to many parts of the world, including Australia, New Zealand, Middle East, South and North Africa and most of the other Mediterranean basin countries. Benson and Walkington (1965) reported that the cultivation of the prickly pear, an Opuntia subgenus, in the United States started with the arrival of the Franciscan fathers who established the

series of missions throughout the coast of Southern California during the eighteenth century. These missions brought with them, from Mexico and Spain, fruit-bearing plants including 2 Opuntia species: O. ficus indica and O. megacantha.

The genus Opuntia is represented by 2 subgenera: Platyopuntia (prickly pear) characterized by flattened pads, and Cylindropuntia (cholla cactus) having cylindrical pads as the name indicates. Opuntia cactus includes many species, some of which are fed to animals or are eaten by humans. Certain species are used for ornamental purposes.

Cactus pads and fruits have been used as food for human consumption for centuries. Indians, Mexicans, Central and South Americans, and North and South Africans have used prickly pear (fruits and young pads) as a staple food in their diet; and some of these populations still do at the present time. Teles (1978) reported that prickly pear pads are sold fresh throughout Mexico and border cities of the United States. According to him, pads are also found preserved in cans and jars.

Opuntia species used for animal nutrition are abundant (within their zone of spread), easy and inexpensive to grow, highly nutritious, palatable and can withstand prolonged droughts (Shoop et al., 1977). Such characteristics make these species a potentially important feed supplement for livestock, particularly during periods of

drought and seasons of low feed availability. The majority of Opuntia plant biomass is pad material rather than fruits. Because of this, livestock feed is derived from cactus pads whose spines are removed. Opuntias are widely fed to cattle, sheep (Shoop et al., 1977) and camels (personal observation). Cactus is served to livestock as fresh forage or stored as silage for later feeding (Castra et al., 1977).

Besides its use for ornamentation and feed, cactus is used for other purposes, too. Natives of desert areas use the strong fiber to make baskets and matting. The spines can be used as fish hooks, combs, tooth picks and as needles or pins. Leather and cloth are sometimes repaired by fastening a slit together by means of cactus spines, which are then held fast by winding twine around them in herringbone fashion (Teles, 1978).

Generally, Platyopuntias (prickly pear) are used as food plants while Cylindropuntias (cholla cactus) are grown for ornamental purposes. However, livestock will eat both types of Opuntias. Little is known about cactus nutrients content, their proportions and their seasonal variability. Past work on the use of Opuntia species for livestock feeding has concentrated on the subgenus Platyopuntia with little attention given to Cylindropuntias. Less information is available concerning the nutritional quality of the pad than is available for the fruit. Moreover, no systematic

survey has been published to document and compare the nutritional qualities of different Opuntia species.

The purpose of this study was to determine the nutritional value of Opuntia pads as forage for livestock and to assess the variability in nutrient content among some North American Opuntia species. A second goal of this study was to determine the seasonal variability of the major pad constituents of a species from Central Oregon (Opuntia fragilis).

LITERATURE REVIEW

1. HISTORIC

Cacti originated from central America and particularly from Mexico. Teles (1978) suggested that the presence of a prickly pear on the Mexican flag undoubtedly indicates the significance of cactus for the citizens of Mexico. Curtin (Teles, 1978) mentioned that some five centuries before the arrival of Cortez about 1325 A.D., the Aztecs ventured into the the region of the present Valley of Mexico where they were defeated by the native inhabitants. While the remaining Aztec invaders were in a marshy spot on the border of a lake, they saw, on a giant rock that rose above the water, a prickly pear upon which was perched a great eagle holding in its beak a struggling snake. The leader of the expedition regarded it as a sign of divine wisdom, and, believing in omens, immediately began the building of Tenochtitlan - or the place of the prickly pear - where the Mexican capitol is now located.

According to Hesse (1973) the two tribes of Indians which were known to have used the prickly pear in the Southwest United States were the Papagos and the Pima. These people were removing the thorns, slicing the pads, and either boiling them in water or frying them in oil until tender. The author insisted on the fact that these people were eating the prickly pear pads, prepared this

way, long before the arrival of Europeans to the American continent.

Griffiths (1905) indicated that it is impossible to tell where and when the feeding of Opuntia cactus to livestock began in the Southwestern United States. However he is certain that the practice was common several years before the civil war. He mentioned that before and after this war, there was very extensive freight transportation of cactus (pads) between some regions in Texas such as Brownsville, Indianola, San Antonio and Eagle Pass.

2. USE OF OPUNTIAS AS A FEEDSTUFF FOR HUMAN AND LIVESTOCK

Opuntia plants have been subject to both praise and condemnation when considered as an alternative feed source. Teles (1978) illustrated this antagonism: While the Mexican was praying there may be no rain when the plants are in bloom so that the fruit may set well and produce a good crop; the legislative assemblies in some Australian colonies were passing laws directed toward the eradication of Opuntias growing in that country. The author also reported that in 1890, while some people in southern Texas were imploring the government to investigate prickly pear eradication, shrewd cattlemen and ingenious machinists were devising means to divest it of its objectionable characteristics at small expense and turn it to a profitable use.

Opuntias as a livestock feedstuff

Opuntias have been cultivated for long time in arid and semi-arid regions as a drought tolerant fodder crop. Their nutritional value, hardiness, ease of cultivation, low establishment and production costs, and high potential yield make it worthy of featuring in agricultural systems (Brutsch, 1979). The work which has been done by Shoop et al. (1977) indicated that cactus pads were a highly nutritious feed for livestock, provided the spines are singed off first (usually through the use of a propane weed burner). Other practices than burning off the spines were discussed by Griffiths (1905). Steaming to moisten the spines and chopping of the big pads were and are, until now, very efficient practices to facilitate the use and maximize the amount of cactus eaten by livestock. The author also indicated that tools and machines have been built for these purposes.

Opuntias are widely fed to camels, sheep and cattle. Singing off the spines is necessary before serving them to the animals, particularly in the case when fed to sheep and cattle. Camels can consume the pads with the spines intact (personal observation). Cactus can be used as fresh forage or as silage (Castra et al., 1977). In a study conducted at the Central Plains Experimental Range near Fort Collins, Colorado, Shoop et al. (1977) found that prickly pear intake depended on the degree of pad burn (to

singe off the spines). Properly singed prickly pear was observed to be a highly palatable forage. They also suggested that prickly pear may have value as an addition to the normal winter feed for short grass ranges in Great Plains. In the same region (near Nunn, Colorado), it was found that heifers fed singed prickly pear pads (O. polyacantha) as a supplement to hay, gained an average of 1.5 pounds daily compared to less than a pound gained by heifers receiving hay alone (USDA, 1976). Woodward (1915) pointed out that when prickly pear was fed with cured fodders or with grains the digestibility of both was increased; and when fed to dairy cattle, milk yield was increased without lowering the percentage of solids in the milk or affecting its flavor.

Opuntias as a food source for humans

Opuntia cactus has several uses and it is known by various names over its distribution zone. Teles (1978) indicated that the prickly pear of the American and Australian, the Indian fig of the Englishman, the Barbary fig of the Frenchman, the tuna of the Spanish American, the higos chumbos of the Spaniards and the nopal of the Mexican are various names of fruit obtained from the platyopuntias growing in these countries.

Most studies of the nutritional value of Opuntia species have emphasized the use of Opuntia fruits as a food

for human populations. The subgenus platyopuntia has the largest use (over the cylindropuntias) and one of its species O. ficus-indica has, as a result, probably been the most extensively studied Opuntia (Teles et al., 1984). From time immemorial, the fig-shaped Sahuaro fruits have been a favorite food for Indian and White alike (Weimer, 1934). This author reported that large quantities of the seedy fruits were eaten fresh, but for the most part, they were made into preserves. The pulp of the fruit was dried in the sun and packed into solid cakes to be stored for winter use. Even the seeds were utilized, being ground and made into nutritive meal cakes. Opuntias use as a fresh (Flath and Takahashi, 1978) or cooked fruit (Teles, 1978), as well as its potential for processing into products such as jam (Sawaya et al., 1983 a) have, also, been reported. After an analysis of the chemical composition of O. ficus-indica seeds, Sawaya and his collaborators (1983 b) suggested that oil meal could be extracted from them.

3. BOTANICAL CONSIDERATIONS

According to Wettstein (Teles, 1978) the botanical classification of Opuntia cactus is as follows:

PHYLUM	= Cormophyta
DIVISION	= Anthophyta
SUBDIVISION	= Angiosperma
CLASS	= Dicotyledones
SUBCLASS	= Choripetalae
ORDER	= Centrosperma
FAMILY	= Cactaceae

GENUS = Opuntia
 SUBGENERA = Platyopuntia
 & Cylindropuntia

Cacti are succulent green-stemmed perennial plants of xeric habitats. Their stems are a series of flattened or cylindrical joints (pads). Stems are fleshy with small ephemeral leaves. Leaves which have been reduced to spines occur in groups at cushionlike areoles where two types of easily detached spines are found, short soft glochids and long stout spines. Some spineless varieties of O. basilaris and O. ficus-indica are available. The flowers are showy, perfect, regular and diurnal. They are born on the areoles of the previous year's growth. Flowers have greenish-yellow, pink, red or white petals joined together in a hypanthium. They have several sepals and numerous stamens also inserted on the hypanthium tube. The ovary is one-celled, inferior, with several fused stigmata. The fruit is a fleshy or dry berry (depending on the species), with many seeds (Marten and Hutchins, 1981).

The genus Opuntia is represented by two subgenera:

- Platyopuntia known as prickly pear and characterized by flattened pads with or without spines.
- Cylindropuntia commonly called cholla cactus and characterized by spiny or spineless cylindrical joints.

In both subgenera, joints are arranged on stems. The number and size of the pads give the morphological appearance of the Opuntia cactus varying from tiny plants to shrubs and even to being tree-like (personal observation). Skilman (1981) pointed out that there are over 250 species in the genus Opuntia.

According to Teles (1978), a rapid and extensive hybridization among the various Opuntia species has increased the difficulties of their systematic taxonomy. Some field studies in California (Benson and Walkington 1965), indicated that cacti are adapted to dry climates and shallow soils, and are usually found on low lands and slopes. The authors also indicated that the cacti population studied was commonly composed of plants varying in characteristics from those of a possible first generation (F1) to those of native species.

4. DAY/NIGHT AND SEASONAL VARIATION IN CACTI METABOLISM

General

Cacti, as other plants of tropical and subtropical zones, possess several ways to adapt themselves to the environment of these regions which is characterized by low rainfall, extreme durnal temperature and high evapotranspiration. Szarek and Ting (1975) pointed out that by the end of the 1800's a variety of succulent plants were

described as demonstrating nocturnal acid synthesis, coupled with diurnal acid catabolism. This unique type of organic acid metabolism became known as Crassulacean Acid Metabolism (CAM), named after the members of the family Crassulaceae in which the metabolism was studied most extensively. In a study conducted by Patten and Dinger (1969) it was stressed that CAM in cacti was interesting in 2 ways: First, the succulent nature of the plant allowed metabolic processes to continue in times of droughts using water stored in mucilagenous cells and secondly, cacti exhibited a form of acid or non-autotrophic CO_2 fixation.

Nisbet and Patten (1974) showed that the CAM in Opuntias permits night-time carboxylation of carbon dioxide (CO_2) to malate, and day-time decarboxylation of malate to CO_2 . These researchers found that CO_2 exchange rates varied seasonally with the temperature and moisture availability. According to Joshi et al. (1965) CAM was considered a significant adaptative mechanism for pineapple and other xerophyte plants allowing them endure the harsh environment under which they grow. The authors indicated that the CAM pathway of CO_2 assimilation permitted atmospheric gas exchange at night when the transpirational water loss was lowest.

Water effects

Acid metabolism and gas exchange studies using two different Opuntia species (O. erinacea and O. basilaris) in two different areas (Washington State and California) showed a significant seasonal pattern in acid metabolism and gas exchange, and that this pattern was controlled by rainfall which significantly influenced the plant water potential, total gas transfer resistances, and nocturnal organic acid synthesis (Littlejohn et al., 1983 ; Szarek et al., 1974). These researchers indicated that in winter and early spring, when plant water stress was mild, stomatal and mesophyll resistances remained low, permitting enhanced nocturnal CO₂ assimilation. The day/night accumulation of acids was large during these seasons. In summer and fall, when plant stress was moderate, the nocturnal assimilation of CO₂ was very low, and the day/night fluctuation in acidity was reduced.

Later work, on the daily trends in CO₂ and H₂O exchange, done by Osmond and his collaborators (1979) using O. ficus indica inermis led to the conclusion that the water content of the cladode influenced stomatal opening and consequently CO₂ transfer. When water content was high, nocturnal stomatal opening resulted in a substantial uptake of CO₂ and synthesis of malic acid during the night. Under water stress, nocturnal stomatal opening

was confined to the latter part of the night and the acid synthesis was reduced by one-third. The same conclusions were drawn by Hanscom and Ting (1978) from their comparative studies done on irrigated and nonirrigated O. basilaris plants: Irrigation magnified CAM photosynthesis while non irrigated Opuntias had maximum acid accumulation after precipitations.

In a study conducted by Szarek and Ting (1975) the day/night variation of titratable acidity in stem tissue was low preceding rainfall, ranging from 5.5 to 12.5 micron eq/cm². At the 5th day after rainfall the acidity reached a maximum of 30.6 micron eq/cm², making an increase of 170%. It was also concluded from this study that CO₂ followed the same pattern with nocturnal CO₂ assimilation averages of 8.8 mg/dm².h and 10.6 mg/dm².h respectively before and after precipitation. Another conclusion was that water stress suppressed gas exchange in the light to a greater extent than in the dark (Neales et al., 1968).

Temperature effects

Most of the studies encountered agreed that water, either from rainfall or irrigation, was the major factor influencing Opuntia pad gas exchange and crassulacean acid metabolism but they did not agree on the importance and specific effects of temperature on these processes.

Nisbet and Patten (1974) found that greatest CO_2 influx rates were at low temperatures while efflux rates were low at higher temperatures. They also indicated that a shift in season caused modifications in CO_2 exchange rates at any one temperature. The months of November through February had the greatest potential for a large net CO_2 influx while the summer months exhibited a small net CO_2 loss. Under a 35/15 degrees C (day/night) thermoperiod, well-watered Opuntia plants supported the fixation of atmospheric CO_2 during the night, early morning and late afternoon. However, under a 20/15 degrees C thermoperiod the CO_2 fixation occurred only during the afternoon (Gerwick et al., 1978). In another study conducted by Hanscom and Ting (1978) it was concluded that maximum acid accumulation in irrigated cacti occurred when there was a maximum difference between day and night temperatures (about 16 degrees C) and when nighttime temperatures were moderate (above 14 degrees C). These researchers indicated that nocturnal acid levels decreased when nighttime temperatures were high (33 degrees C in August) or cool (below 12 degrees in December - February). However, Osmond (1979) pointed out that nighttime temperature had little effect on gas exchange and acid synthesis.

Parallel to seasonal trends in metabolism, cacti show seasonal dry matter variability. In research conducted in the short grass region of the Great Plains,

Shoop et al. (1977) indicated that the dry matter content of prickly pear was lowest in June (14.6%) and highest in February (45.8%).

Explanations of the processes

As an explanation of the phenomenon of CO₂ and water vapor fluxes control and their consequences in xerophyte and mesophyte plants, Neales et al. (1968) indicated that low transpiration rates of xerophytes in light could be caused by either a low and varying water vapor pressure in their leaves or by a higher diffusive resistance than the one of mesophyte leaves. These researchers indicated that the control of stomatal aperture in both succulents and nonsucculents was affected by variations of internal CO₂ concentration in the leaf. A difference in the rhythm of stomatal aperture between these two kinds of plants could be related to the dark carboxylation mechanism of succulents that does not exist in the nonsucculents. Szarek et al., (1973) formulated the following hypothesis concerning the metabolic regimes of dry/wet periods and day/night cycles: The endogenously produced CO₂ was retained and recycled through dark fixation, organic acid transformations, photosynthesis and respiration.

We can summarize the above results with a conclusion drawn from a global study of these processes conducted by Nobel (1977) on O. basilaris: The enhanced stomatal

opening during cool nights, the reduced transpirational water loss, and dark CO₂ fixation combine to maximize the efficiency of water use (mass CO₂ fixed/mass H₂O transpired) for Opuntias and other CAM plants. These physiologic and metabolic processes are an important adaptive strategy for arid and semi-arid habitats.

5. GROWTH AND YIELD

Growth

Analyzing the growth form aspects of O. compressa as a function of canopy cover and soil type, Abrahamson and Rubinstein (1976) showed that the former factor was more important in determining the growth form of the plant. Clustered plants were significantly taller than the ones found in open areas, and individuals growing clustered had fewer pads than those grown in the open, independently of soil type.

At the Central Plains Experimental Range, Fort Collins, Colorado, Bement (1968) studied the effects of grazing intensity, using cattle, on prickly pear growth and yield. He reported that the increase was largest under no grazing, intermediate under moderate use and least under heavy grazing.

Another factor that might influence growth is CAM, as discussed above. According to Neales et al. (1978), CAM of succulents could be an important factor influencing

their growth: The restriction of transpiration limited the potential influx of carbon dioxide (CO₂) into leaves, thus reduced the acid synthesis and consequently plant growth. However, the CAM was viewed by Osmond and his collaborators (1979) only as a photosynthetic pathway and not noted for high productivity but rather for persistence in arid habitats.

Yield

Woodward (1915) emphasized the low cost of growing cactus in relation to the tonnage produced. Le Houerou et al., (1965) indicated that green yields of O. ficus-indica (inermis) ranging from 15 to 100 metric tons/ha/year were obtained in Tunisia. The author related this variability to differences in soil types and amount of rainfall in regions where production was measured. According to Shankar et al. (1976), yields ranging from 10 to 30 tons/ha/year of green fodder have been obtained in Mediterranean areas and 12.5 tons/ha in Southern India. Fresh yields of 50 and 93 metric tons/ha/year were reported from Tunisia and Brazil (Acevedo et al., 1983).

6. MAJOR OPUNTIA PAD CONSTITUENTS

Opuntia species have been analyzed for nutrient content as early as 1912. Ranchers as well as researchers were at that time, as now, interested in the use of

Opuntias as an alternative feed. Results of early investigations done by Hoffman and Walker (1912) into the nutritive quality of cactus are presented in table 1. The more recent findings are shown in tables 2, 3, 4 and 5.

Table 1: Percent Chemical Composition (average - DM basis) of O. engelmannii and O. Lindhermeri (Hoffman & Walker, 1912)

- Water	: 85		
- Crude protein	: 1.4-4.4	- Phosphoric acid	: 0.33
- Nitro. Free Extr.	: 7.85	- Potash	: 3.04
- Fat	: 1.55	- Magnesium	: 1.6
- Crude fiber	: 8.65	- Calcium	: 2.84-13.85

One of these early investigations also indicated that spiny and spineless Opuntias have practically the same chemical composition and are of equal value for feeding purposes (Woodward, 1915).

In a comparative study (table 2) between oven-dried prickly pear (O. polyacantha), sun-cured grass hay of Agropyron elongatum and Bromus inermis (over ripe), and sun-cured hay of Medicago sativa (early bloom), Shoop et al. (1977) found that the prickly pear contained only 3.4% digestible protein which is about equal to that of the grass-hay pellets but only one third that of the alfalfa hay. They concluded also that a ration including prickly pear might benefit from protein supplements. On the other hand, the analysis indicated that prickly pear had high levels of soluble carbohydrates making it a readily available source of energy (2.6 Mcal of digestible energy per kilogram dry matter in O. polyacantha).

Table 2 : Percent Chemical Composition (1) of Prickly pear, Alfalfa Hay and Pelleted Grass Hay (Shoop et al., 1977)

Constituents	alfalfa hay	prickly pear	prickly pear compared to alfalfa (2)	grass-hay pellets
Total ash	7.5b	13.5a	80% more	7.5b
NDF	39.6b	34.0c	14% less	63.1c
ADF	32.6b	23.3c	30% less	41.6a
Lignin	9.5a	7.8b	20% less	8.1b
Soluble portion	60.5b	66.0a	10% more	36.9c
Hemicellulose	7.0c	10.7b	53% more	21.5a
Cellulose	15.6b	2.0c	85% less	26.1a
Soluble carbohydrates	43.7b	60.6a	38% more	31.2c

- (1) Means in the same line followed by different letters differ significantly at 5% level.
 (2) Comparison made from the data given by Shoop et al. (1977)

Le Houerou and his collaborators (1965) (tables 3 and 4) indicated that Opuntia pad, as a feed source, are thought to be an unbalanced feed for livestock. They stated that Opuntia pads are low in protein and fats, high in water, carbohydrates and vitamins, with a very high calcium/phosphorus ratio. These authors reported that the proportions of protein, fat and nitrogen free extract decreased with the age of the plant inversely to cellulose and lignin contents which increased. Some other data about prickly pear content are presented in table 6 as reported by Teles (1978).

Table 3 : Pad Composition (% , on Green Matter Basis) of O. ficus-indica inermis Grown in Tunisia (Le Houerou et al., 1965).

Age (Years)	Dry Matter	Crude Fiber	Crude Protein	Ether Extract	NFE *
<1	11.04	1.03	0.52	0.17	8.10
1	9.83	1.10	0.50	0.13	5.48
2	12.32	1.66	0.45	0.15	7.89
3	14.91	2.29	0.40	0.16	8.80
4	15.30	3.17	0.44	0.15	8.20
5	11.75	2.64	0.40	0.14	6.00

* NFE = Nitrogen Free Extract

Concerning the mineral composition, Shoop et al. (1977), working on O. polyacantha, indicated that the phosphorus content was below livestock dietary requirements. Calcium levels seemed to be adequate but the calcium-phosphorus ratio, of about 36/1 was too high for optimum livestock performance. According to the same source, the other minerals (manganese, copper, zinc, magnesium and iron) had concentrations within the range generally suggested to be acceptable in ruminant diets. An exception was sodium content which was relatively low (.02%). However, these researchers indicated that sodium deficiency would not be an important problem since that mineral could be routinely provided in a salt supplement.

Le Houerou and his collaborators (1965) also conducted some mineral analyses on prickly pear pads. The results obtained are presented in table 6.

Table 4: Nutrient Content of *O. ficus-indica inermis* on Green and Dry Matter (Le Houerou et al., 1965).

		De Cock (1965)	Theriez (1965)	Monjauze (1964)	Lozano (1958)	Mourisson (1957)
MOISTURE		89.56	90.20	—	92.05	87.40
CRUDE PROTEIN	Green	0.38	—	—	0.52	0.90
	Dry	—	2.50	7.87	—	6.96
CRUDE FIBER	Green	—	—	—	0.90	0.40
	Dry	—	14.50	13.25	—	9.30
TOTAL LIPIDS	Green	0.16	—	—	0.09	0.40
	Dry	—	—	2.00	—	3.10
N. FREE EXTRACT	Green	6.62	—	—	5.61	7.80
	Dry	—	—	56.70	—	60.29
ASH	Green	2.03	—	—	1.08	2.60
	Dry	—	17.80	17.80	—	20.15

* All the studies were done on plants of 1 to 2 years old.

Table 5: Major Prickly Pear Content (%) on Green and Dry Matter basis (Teles, 1978).

		Teles Church & McDowell Church			INCPA	USDA
		(1978)	(1975)	(1974)	(1961)	(1920)
MOISTURE		95.26	85.00	83.00	88.90	89.40
CRUDE PROTEIN	Green	0.87	0.50	0.66	1.10	0.35
	Dry	4.82	3.33	3.88	10.00	3.30
CRUDE FIBER	Green	1.61	1.60	1.33	2.60	1.22
	Dry	8.94	10.60	7.82	23.26	11.51
TOTAL LIPIDS	Green	0.42	0.10	1.11	0.40	0.23
	Dry	2.48	0.67	6.53	3.64	2.17
CARBO-HYDRATES	Green	11.86	10.90	10.02	11.02	7.21
	Dry	65.88	72.67	64.82	64.82	68.06
MINERAL ASH	Green	1.12	—	0.90	1.60	2.89
	Dry	23.53	—	8.18	15.10	17.00

Table 6: Mineral composition (% - DM basis) of pads of O. ficus-indica inermis (Le Houerou et al., 1965)

- Manganese	: 0.43	- Potash	: 9.70
- Calcium	: 27.40	- Phosphorus	: 0.40
- Magnesium	: 5.33	- Iron	: 0.30
- Sulfur	: 0.52	- Silicon	: 0.58
- Chloride	: 1.84	- Actinium	: 0.23

7. OPUNTIA PAD DIGESTIBILITY

A report of US Department of Agriculture (Anonymous, 1976) indicated that feeding tests, using heifers, rated cactus as more readily and more completely digestible than grass-hay (Agropyron cristatum and Bromus spp.). In a study conducted by De W. Rossouw (1961) a comparison, of yield and amount of digestible portion, between prickly pear and some other fodders is summarized in table 7.

Table 7: Total Yield and Amount (as fed) of Digestible Nutrients of Some Fodders (De W. Rossouw, 1961)

Crop	Yield (t./ha)	Amount of digestible nutrients (t./ha)	%
Prickly pear	80	5	6.25
Maize (silage)	25	4.2	16.80
Mangelwrizel	25	3.7	14.80
Lucerne hay	5	2.5	50.00

According to Shoop et al. (1977) 80% of the total digestion of Great Plains prickly pear (O. polyacantha) occurred during the first 16 hours of a 48-hour incubation period whereas only 73% and 71% of total digestion for hay pellets and alfalfa hay, respectively, occurred during the

initial 16 hours. Comparative dry matter digestibilities (In vivo - nylon bag - and In vitro) of the three forages are shown in table 8.

Table 8 : Dry Matter Digestibility (%) In vivo (NBDMD) and In vitro (IVDMD) of Singed Prickly pear, Grass-hay Pellets and Alfalfa (Shoop et al., 1977)

Feed	N B D M D		I V D M D	
	16 hour incubation	48 hour incubation	96 hour incubation	
Prickly pear	52.9 a	66.4 a	63.8 a	
Grass-hay pellets	39.3 c	54.1 c	53.0 b	
Alfalfa hay	44.5 b	62.9 b	63.7 a	

N.B. Means in the same column followed by different letters differ significantly at 5% level.

A rapid rate of digestion means a faster passage of the material through the digestive tract. This could lead to increased feed intake by the animal and consequently improved rate of live weight gain (particularly when the ration is balanced).

8. TRIALS TO INCREASE FEED VALUE OF OPUNTIA SPECIES

Opuntia cactus can be stored for a later use during the dry season. Castra et al. (1977) conducted an experiment to determine the feasibility of preserving prickly pear using some additives. Cactus silage was evaluated in

terms of its nutritive value, odor, color and acceptability by lambs. Cactus was chopped and equal amounts were hand-mixed and stored in plastic bags for 45 days with each of the additives. The additives were: 5% Urea, 8% urea-molasses (5% urea + 3% cane molasses), 15% ground sorghum grain or 15% dried poultry waste. The best additives for reducing losses during storage and for maintaining the palatability of silage were sorghum-grain and urea-molasses. Dried poultry waste affected the color and the odor of the silage whereas the urea liquified the silage mass (only the coarse fichers were left intact). Both of these additives reduced the acceptability of the resulting silage to lambs. In a subsequent experiment Castra and his collaborators (1977) tried to determine the nutritive value of prickly pear silage and assess the possibility of increasing dry matter and protein content. Cactus pads (200 Kg per treatment) were ensiled using for each, one of the following additives: 8% urea-molasses, 15% sorghum grain, and 15% sunflower meal. Mixtures were stored in metal containers for 60 days. All additives significantly ($P < 0.05$) increased the dry matter and the protein content of the silages. The results of the analyses are presented in table 9. Gross energy was significantly increased by sorghum-grain and sunflower meal but not by urea-molasses compared to silage made without an additive.

Table 9: Proximate Analysis (Dry Matter Basis) of O. ficus-indica (Thornless Prickly pear) silages using some additives (Castra et al., 1977)

Item (%)	Control	Additive Treatments		
		Sorghum	Sunflower	Urea-molas.
dry matter	8.2 d	16.8 b	19.5 a	11.4 c
organic mat.	86.8 b	93.2 a	91.3 a	87.7 b
crude protein	5.2 d	9.1 c	25.7 a	21.4 b
ether extract	1.9 b	3.6 a	1.3 b	1.8 b
crude fiber	15.5 b	15.5 b	26.5 a	17.0 b
N F E	57.9 b	64.9 a	37.7 d	47.4 c

Means on the same line with different subscript are significantly different at 5% level.

The acceptability of the silage was tested by feeding it to 12 lambs for 7 days. The prickly pear-sunflower was the most palatable but the least digestible of the different mixtures studied (table 10).

Table 10: Consumption and In vivo Digestibility of O. ficus indica Silages Using Some Additives (Castra et al., 1977)

Item	Control	Sorghum	Sunflower	Urea-molas
dry matter intake (Kg)	1.404	2.575	3.097	2.002
D.M. digestibility (%)	70.40	77.50	65.10	81.20
Weight change (Kg)	-3.400	-1.600	-.900	-2.100

From these series of experiments Castra et al. (1977) concluded that prickly pear silage could be a valuable

feed resource for animal producers in arid or semi arid regions, particularly during the dry season.

In a work on prickly pear, 'white' variety, done by Belasco et al. (1958), urea was used as a foliar treatment in the range and in the laboratory. In the laboratory, uniform pads were dipped for 5 seconds in a urea solution composed of 200 lb. of urea, and a combination of wetting and adhesive agents per 100 gal. of water. The control pads were dipped in distilled water for the same period of time. The pads were then dried in an oven at 150 degrees F for 24 hours prior to analysis. In the field test, urea was spread on stands of the same variety as above using 160 lb. of urea per 40 gal. of water per acre (a spreader-sticker was used). The control area received no urea. Samples for analyses were collected 7 days later. For both trials (in the laboratory and field) the nitrogen content of the prickly pear pads increased but not significantly (at 5% level). The In vitro dry matter and cellulose digestibility rates increased significantly (at 5% level). The authors also indicated that these treatments did not affect the morphological state of the pads.

VEGETATIVE MATERIAL
AND
EXPERIMENTAL METHODS

1. VEGETATIVE MATERIAL

The study had two major goals, the first was pad content analysis to document the nutrient characteristics of several different Opuntia species (from the Southwestern United States) as a feed for domestic livestock. The second goal was a survey of nutrient seasonal variations of a species: Opuntia fragilis from Central Oregon (John Day National Fossil Beds - Mitchell, Prineville).

For the species nutritive content comparison, four platyopuntia (prickly pear) and one cylindropuntia (cholla cactus) species were studied. The platyopuntia species analyzed were: O. fragilis, O. polyacantha, O. filipendula, O. engelmannii (also called O. phaecantha or Common prickly pear). The cylindropuntia included in the test was O. versicolor. Cactus pads were solicited from colleagues around the western United States:

- Central Oregon → O. fragilis (collected on 5/15/86)
- New Mexico → O. filipendula (collected on 06/8/86)
- Arizona { → O. engelmannii (collected on 6/30/86)
- O. versicolor (same time and site)
- Colorado → O. polyacantha (collected on 6/20/86)

In an attempt to reduce seasonal effects among species it was requested that all material would be collected during the same period of time (May-June 1986).

Opuntia fragilis samples were collected by myself. Several plants were included in each collection. In order to minimize variability between seasonal collections, samples were collected from the same plants in each season. The collections took place on the following dates: February 7th, May 15th, August 19th and November 5th of 1986.

Each species was analyzed for Neutral Detergent Fiber (NDF), Acid Detergent Fiber (NDF), lignin, cellulose, hemicellulose, acid insoluble ash, crude protein, ash, phosphorus, calcium, gross energy, In vitro Dry Matter Digestibility (IVDMD), and In vivo dry matter and crude protein disappearance. For reference and comparison purposes, parallel analyses of alfalfa (Medicago sativa) hay and grass (Agropyron cristatum and Bromus spp.) hay samples were included in each run.

The comparison between the Opuntia species relied only on the results as obtained from the laboratory analyses. This was due to:

- The growing conditions of the plant species included in the analyses and their relative age at the time of collection were not known.

- Limited time allocated to the present work that made a deep physiological study of cactus impossible.

2. SAMPLING

Opuntia pads, from the different species included in the analyses, were received fresh. Immediately upon reception the plant vegetative material was dried at 50 degrees C. in a forced air oven to a constant final weight (dry); This temperature was chosen to avoid destruction of organic matter and chemical transformations. The samples were then ground to pass through a 1 mm. screen (30 mesh) and stored in sealed plastic bags.

3. METHODS

FIBER CONTENT

The crude fiber components were analyzed according to the methods described by Goering and Van Soest (1970) in the Agricultural Hand Book No. 379 (Forage Fiber Analysis) modified by Waldern (1971) through the use of the metallic block for refluxing, besides the reduction in sample size (0.35 g) and reagents quantities.

Four subsamples from each species were used in the fiber components determination. In order to minimize variability inherent to experimental design, all the samples were analyzed in the same time and under the same conditions. The analyses were done sequentially as recommended by Van Soest and Robertson (1977, 1980) and Marten (1981). This sequential extraction technique removed

the interference of pectin, tannins, and silica allowing a more accurate estimate, particularly, of hemicellulose and lignin. The analysis was performed in this manner because the neutral detergent solution dissolves pectin, tannins and sometimes silica whereas acid detergent does not dissolve all the pectins, the tannin-protein complex, or the silica. However, cell wall proteins were largely dissolved by acid detergent and not by neutral detergent solution. Thus, in order to obtain a purified ADF, the neutral-detergent extraction preceded that of acid detergent fiber.

Neutral Detergent Fiber

The Neutral Detergent Fiber (NDF) is the insoluble portion left after action of a neutral detergent solution on the sample (0.35 g) of Opuntia pads following the method of Goering and Van Soest (1970) as modified by McQueen and Nicholson (1979) through the use of amylase (enzyme prepared from *Bacillus subtilus* type III A) to eliminate starch from the remaining NDF and thus determine the proportion of the latter more accurately. After alternate and repeated washings with hot water and acetone followed by overnight drying at 100 degrees Celsius, the remaining NDF was reported as percent cell wall constituents.

Acid Detergent Fiber

The ADF extraction used the remaining NDF according to the Goering and Van Soest procedure (1970). The reagents added to the NDF residue were the acid detergent solution followed by the decahydronaphthalene (reagent grade). After filtration, washing with hot water then acetone and oven drying at 100 degrees Celsius for 8 hours the residual material was reported as the ADF content.

Hemicellulose

The hemicellulose portion of plants is estimated as the difference between the cell wall (NDF) and ADF components as was recommended by Goering and Van Soest (1970).

Lignin and cellulose

For the lignin extraction two experimental procedures were described by Goering and Van Soest (1970): Permanganate lignin and Acid-detergent Lignin (using 72 % sulfuric acid). The permanganate method was used because it has several advantages over the 72% Sulfuric Acid procedure particularly by making possible the determination of cellulose content through a sequential extraction. The residual Acid Detergent Fiber (ADF) served as a preparatory step. The lignin portion was oxidized with an excess of acetic acid-buffered potassium permanganate solution (2:1) using trivalent iron and monovalent silver as

catalysts. Lignin content was determined as the loss of weight from the initial weight of ADF.

An overnight ashing of the residual yielded cellulose content as the weight loss.

CRUDE PROTEIN

The crude protein was analyzed following the Copper Catalyst Kjeldahl Micro Method (A.O.A.C., 1984). Samples were run duplicate and under the same experimental conditions. These samples were digested with sulfuric acid using copper sulfate as a catalyst. Plant sample nitrogen was converted to ammonia that was distilled and titrated.

MINERALS: PHOSPHORUS AND CALCIUM

Analysis of phosphorus and calcium was carried-out because of their importance in animal feeding. The analyses were accomplished according to the A.O.A.C. methods (1975). Samples were run in duplicate. One gram-samples were ashed at a temperature of 500 degrees C then predigested with concentrated hydrochloric acid (Hcl _ 5 N). A final digestion occurred using 0.1 N of hydrochloric acid. On this aliquot, phosphorus content was determined following the Vanadomolybdate procedure using a regular spectrophotometer and calcium portion using the Atomic Absorption spectroscopy.

GROSS ENERGY

Gross energy was determined using the bomb calorimeter. The analysis was accomplished according to the operating instructions prepared by M. Goeger and revised in April 1986. Oven dried samples of Opuntia pads were ignited and allowed to combust completely in the bomb chamber. The resulting heat from this combustion was transferred to the surrounding water, of known initial temperature, through the metal of the bomb chamber. The water temperature rise, net weight of the sample (on a DM basis), the length of the portion of fuse wire burned, the quantity of acid used for titration and the energy equivalent of the calorimeter (constant for the whole analysis) were used to calculate the gross energy content of the samples.

THE TWO-STAGE IN VITRO DRY MATTER FERMENTATION

The procedure applied was essentially that of Tilly and Terry (1963) modified by a direct acidification at the end of the first stage for pH adjustment and a filtration at the end of the second stage as suggested by Alexander and McGowan (1966). The two stages were:

- Stage 1: Incubation with rumen liquor. A rumen digesta was provided by a rumen-fistulated cow (one of the cows used to test the In vivo dry matter and crude protein

digestibility). The filtration of the digesta through a cheese cloth gave the liquor that was added, with a synthetic saliva (McDougall's buffer solution), to the cactus samples. The whole was incubated, during 48 hours, at a temperature of 38 degrees C and in an anaerobic state using the carbon dioxide.

- Stage 2: Addition of pepsin. The introduction of pepsin was intended to remove the undigested protein. After the addition of pepsin solution to the digesta, another incubation for the same period and under the same conditions as in stage 1 started. At the end of this second stage the digesta was filtered and the residue was dried overnight. The results were used to calculate the percent In Vitro Dry Matter Digestibility (% IVDMD) as the weight loss due to digestion of Opuntias dry matter.

IN VIVO DRY MATTER AND CRUDE PROTEIN DIGESTIBILITY

The analyses were designed according to the procedure described by Mehrez and Orskov (1977) and following the recommendations of Stern and Sater (1984) and Nocek (1985). The technique used small bags (17 x 9 cm) containing 5 gram-samples placed in the rumen of a fistulated steer. This technique measured the disappearance of feed constituents, in this case Dry Matter (DM) and Crude Protein (CP), from the bags suspended in the rumen for a predetermined length of time. The procedure was the same

for the determination of DM and CP digestibilities with 2 exceptions:

- The incubation period of bags in the rumen was 24 and 48 hours in the case of DM and only 6 and 12 hours in the case of CP.

- In the case of DM digestibility determination, the residue was dried at a 100 degrees C to determine its weight on a Dry Matter basis (this step was accomplished after initial drying following the removal of the bags from the rumen). In the case of Nitrogen disappearance this "first drying" was followed by an analysis of the crude protein content of the residue according to the Copper Catalyst Kjeldahl Micro Method (A.O.A.C. Journal, 1984).

Five rumen fistulated cows were used in the experimentation. These cows were maintained on medium quality grass hay with mineral supplements (ad libitum) for a preadjustment period of 10 days and during the experimentation. The 2 types of analyses for determination of DM and CP digestibilities were done separately. Three cows were used to determine CP disappearance and five cows in the case of Dry Matter.

The digestion bags were made from a nylon cloth of a 53 microns-pore size as was recommended by Dr. James Carpenter (personal communication), Mehrez and Orskov (1976), Stern and Sater (1984), and Nocek (1985). The

bags were double sewn and a water proof-glue was applied on the sewing lines to seal the needle holes as recommended by Van Dyne (1962). The nylon bags, of dimensions: 17 x 9 cm, and containing a 5 g sample, each, were suspended in the cow rumen through the fistula. According to Mehrez and Orskov (1976) a bag of the dimensions indicated above was adequate for incubation of 5 g air dry feed. The bags were tied separately with nylon string (length = 40 cm). The other end of the string was tied to a wire hooked to the top of the cannula (each block of bags was hooked separately). An anchor weighing about 15 g was tied to the bottom of each bag to prevent it from floating. After being soaked in the water for about 1 min. the bags were embedded in the rumen digesta. In order to reduce the variability between the 48 hours group of bags and the one for 24 hours, the 2 groups were put at different times and removed in the same time. The group of bags to be incubated for 48 hours were put in the rumen first then the group to stay only 24 hours. The same plan of succession was applied in the case of crude protein digestibility: the 12 hours group of bags were placed first followed by the 6 hours group. This was done in order to have the bags, from the same animal, washed under the same conditions as was recommended by Van Dyne (1962) and Mehrez and Orskov (1976). After being washed under running tap water, the bags and their contents were

allowed to dry at 50 degrees C to a constant weight. This step was followed by determination of DM and CP as described above.

RESULTS AND DISCUSSION

This study was oriented toward the nutrient composition of several cactus species from the Southwestern United States and the seasonal variability of these nutrients in O. fragilis. The comparisons, herein reported, were based only on the results as obtained from the laboratory analyses. All calculations were done on a Dry Matter basis.

The first part of this chapter focuses on the determination of differences between nutrient content of the species included in the analyses. The second part reports the seasonal variability of these components in one of the species, O. fragilis.

SECTION I: SPECIES COMPARISON

1. CRUDE FIBERNEUTRAL AND ACID DETERGENT FIBER

Forage intake is mainly controlled by physical factors of the feed particularly its fibrous components (lignin, cellulose and hemicellulose), and their rumen retention time. Marten (1981) reported that new hay grading standards proposed by the American Forage and Grassland Council depend upon use of the acid detergent fiber for estimation of digestibility, and neutral detergent fiber for estimation of potential intake of legumes and grasses.

Table 11: Average Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) Proportions (% DM basis) of the Opuntia Species.

Species	NDF	ADF
Alfalfa (ref. sp)	45.15	29.91
<i>O. engelmannii</i>	31.18	11.29
<i>O. filipendula</i>	33.30	15.31
<i>O. versicolor</i>	39.85	18.98
<i>O. polyacantha</i>	31.16	18.42
<i>O. fragilis</i>	35.08	15.47

Neutral Detergent Fiber

The Neutral Detergent Fiber procedure (NDF) was used to separate the samples the dry matter into the readily-available soluble constituents (soluble portion) and those

that are incompletely available and are dependent on a microbial fermentation (Neutral Detergent Fiber portion or Cell Wall Components). The results of this NDF analysis are presented in table 11. Similar proportions were found by Shoop et al. (1977).

Because of the sticky character of cactus pad mucilage, Neutral Detergent Fiber (NDF) filtration through crucibles (pore size = 50 microns), was very difficult and lasted longer than that of alfalfa samples used as reference. This phenomenon was particularly experienced with O. fragilis, O. versicolor and O. polyacantha samples. Pigden (1980) indicated that one of the main problems of filtering Neutral Detergent Fiber is starch that tends to form viscous solutions in hot neutral-detergent.

From table 11 it appears that the proportion of Cell Wall Constituents (NDF) in Opuntia samples was relatively low when compared to that of alfalfa. This proportion would be significantly lower, particularly for O. fragilis, if the spines were removed before analysis. The presence of spines increases crude fiber content and, consequently, reduces intake and dry matter digestibility of cactus pads. Livestock, usually, eats Opuntias with or without spines. Singing off these spines, when present, would increase both intake and digestibility leading to higher livestock performance.

Acid Detergent Fiber

The Acid Detergent Fiber (ADF) portion of the sample was determined by a sequential procedure using the Neutral Detergent Fiber residue in order to obtain more accurate estimates of ADF, lignin, cellulose and hemicellulose. This approach avoided interference of pectins and tannins in ADF analysis, as was recommended by Van Soest and Robertson (1980). The mucilaginous problem encountered in NDF extraction did not interfere with the ADF analysis. It could be concluded that the pad mucilage might be formed of tannins, pectins and certain types of starch that were dissolved by the neutral detergent solution and amylase used in the Neutral Detergent Fiber residue extraction. The ADF proportions are presented in table 11.

From table 11 it appears that the ADF residue of Opuntias was low comparatively to that of alfalfa samples, particularly in the case of O. engelmannii and O. filipendula. This relatively low ADF proportion should give them an appreciable digestibility level especially when spines are singed off.

HEMICELLULOSE, CELLULOSE AND LIGNIN

Hemicellulose, cellulose and lignin were determined through sequential extractions. This procedure increased the accuracy of proportions determination mainly in obtaining a ligno-cellulosic residue free from tannins and

pectins. Table 12 summarizes the content of the above components in Opuntia samples.

Table 12: Average Percent Hemicellulose Cellulose and Lignin Content (DM basis) of the Opuntia species Analyzed.

Species	Hemicellulose	Cellulose	Lignin
Alfalfa (ref. sp)	15.24	21.49	7.93
<u>O. engelmannii</u>	19.88	7.95	2.89
<u>O. filipendula</u>	17.99	10.49	3.97
<u>O. versicolor</u>	20.87	13.73	3.86
<u>O. polyacantha</u>	12.74	12.69	4.79
<u>O. fragilis</u>	19.61	10.97	3.91

Hemicellulose

The hemicellulose portion was obtained by subtracting the Acid Detergent Fiber residue from the Neutral Detergent Fiber portion in the sample as recommended by Goering and Van Soest (1970), Van Soest and Robertson (1980) and Marten (1981). Hemicellulose content is presented in table 12. O. versicolor had the highest proportion (20.87%) and O. polyacantha had the lowest (12.74%). The latter species, having the second highest content but the lowest hemicellulose and thus high cellulose and lignin proportions. These two nutrients being the least digestible fiber components, would affect considerably the digestibility level of that species, O. polyacantha. It appears from table 12 that the nutrient quantities eliminated by the acid solution (hemicellulose, cell wall

proteins and others) were relatively important and could contain high tannins and pectins proportions.

Cellulose

Opuntia samples showed a relatively lower cellulose content if compared to that of the reference samples (table 12). In all cases the cellulose content was about two-third of ADF proportions (lignins counted for less than one-third). This should raise the digestibility level because, compared to lignin, cellulose is more digestible. The In vitro and In vivo analyses did not show the expected rates in the case of cactus samples comparatively to that of alfalfa if expectations based only on fiber content. Cellulose proportions as obtained from the analysis are presented in table 12.

Lignin

The lignin content of feeds determines, to a large extent, their nutritive values because it is the least indigestible nutrients particularly when large proportion of silica is present in the feed (Marten, 1981 ; Van Soest, 1982). The association of lignin with cell wall proteins lowers drastically their digestibility (Pigden, 1980). Even though O. polyacantha was the second highest in ADF and cellulose contents, it was the least digestible among the Opuntia samples (In vitro and In vivo DM

digestibility) certainly because of its higher lignin content (table 12).

Conclusion

From the above tables (11 and 12) it appears that Opuntias have a relatively reduced fiber content in comparison to alfalfa. This characteristic of low fibrous portion in Opuntias, as in other high moisture-containing plants, has been documented in other literature (Le Houerou et al., 1965; Teles, 1978 and Shoop et al., 1977). The analyses indicated that alfalfa samples had a higher ADF, cellulose and lignin content than cactus species which were higher in Neutral and Acid Detergent Fiber soluble portions. Further analyses are needed to be done in order to determine the components of these soluble portions and their concentrations. Among the Opuntia species, O. versicolor and O. polyacantha had the highest fiber proportions, and O. engelmannii had the lowest. The In vitro and In vivo analyses showed logical inverse results. The digestibility rates were respectively 50.48% (In vitro) and 48.44% (In vivo) for O. versicolor, 44.48% and 44.61% in the case of O. versicolor, and 59.22% and 57.22% for O. engelmannii. Even though alfalfa samples were higher than those of cactus in different fiber components, they were higher in dry matter disappearance rates. Thus, the digestibility was not only related to

fiber content but other factors, acting together or on individual basis, might interfered. Concerning Opuntia samples, these factors might be the mucilagenous character, the acidity of Opuntias cell content, besides the possible high proportions of tannins and pectins. Generally speaking, the low fiber content of Opuntia species makes them fit in rations including other high fiber feeds which might increase the ration digestibility level. In addition, cactus high moisture content allows livestock, in arid and semi aride regions, to extract from it a large part of their water requirements.

2. CRUDE PROTEIN

Nitrogen content of feedstuff is one of the most important criteria used to determine the nutritive value of forages and the expected weight gain when these forages are fed to livestock. The crude protein content of the Opuntia species , as determined by the Copper Atalyst Kjeldahl Micro Method (A.O.A.C., 1984), was as shown in Table 13.

Table 13 shows that species relative contents are very different from that of alfalfa and grass hay samples. This could be related to growing site characteristics: Cacti grow, usually, on slopes and poor soils (Kinraide, 1978). Physiological and genetic differences might be taken into consideration also.

Table 13: Average Crude Protein content (% DM basis) of the Opuntia Species Included in the comparative study.

Species	Mean
Alfalfa (ref. sp)	18.29
Grass hay (ref.)	8.84
O. engelmannii	4.16
O. filipendula	2.95
O. versicolor	6.83
O. polyacantha	3.61
O. fragilis	3.44

As was reported in the literature by Le Houerou and his collaborators (1965), Castra (1977), Shoop et al. (1977) and Teles (1978), the samples analyzed during this study showed that Opuntia samples were low in crude protein content. According to Shoop et al. (1977), the crude protein concentration of prickly pear was less than 1/3 that of alfalfa hay, but about equal to that of grass hay.

This low crude protein content of Opuntias should be taken into consideration when formulating rations for livestock feeding. A ration which includes an appreciable proportion of cactus pads should be supplemented with a feed relatively high in protein content.

3. MINERALS: PHOSPHORUS AND CALCIUM

Analyses of phosphorus and calcium was carried-out because of their importance in animal feeding. The

analyses were run according to the A.O.A.C. procedures (1975). The results are presented in table 14.

Table 14: Phosphorus and Calcium Levels (% - DM basis) in Pads of the Opuntia Species (means).

Species	Phosphorus	Calcium	Ca/P
Alfalfa (ref. sp)	0.19	1.68	9
<u>O. engelmannii</u>	0.16	5.79	30
<u>O. filipendula</u>	0.17	9.71	64
<u>O. versicolor</u>	0.08	6.21	77
<u>O. polyacantha</u>	0.16	6.77	42
<u>O. fragilis</u>	0.29	6.33	29

Phosphorus

Spectroscopy analysis indicated that, except for O. fragilis, cactus samples were low in phosphorus content. This was mainly due to phosphorus deficiencies of the soils where Opuntias usually grow. The analysis results (table 14) show that most of the Opuntias included in the study had phosphorus levels below growing and breeding cattle requirements ranging from 0.17 to 0.59% (NRC, 1984). This phosphorus deficiency in Opuntia species should be taken into considerations when formulating live-stock rations including important quantity of cactus.

Calcium

Generally, arid and semi-arid soils contain important quantities of calcium compounds and thus the atomic

absorption spectroscopy resulted in very high calcium concentration in Opuntia samples as compared to that of alfalfa hay (reference). In addition to this high soil calcium content, the water deficiency pushes the cactus plants growing under these conditions to accumulate in their pads the highest possible quantity of solutes, mainly calcium. This process allows the plant to extract, through osmosis, as much water as possible from the soil. In the studied cactus species, calcium levels largely exceeded the requirements of growing and breeding cattle ranging from 0.17 to 1.59% of Dry Matter content (NRC, 1984).

Conclusion

Similar trend of Opuntias, as having low phosphorus content and high calcium levels, was reported in the few literature found on mineral content of cacti. Shoop and his collaborators (1977), working on O. polyacantha, stated that the phosphorus content was below livestock dietary requirements. These authors, also, indicated that the calcium level seemed to be adequate but the Calcium/Phosphorus ratio, of about 36/1, was too low for optimum livestock performance. The mineral analyses of the five Opuntia species included in this study resulted in ratios ranging from 30/1 to 77/1 (table 14).

4. TOTAL ASH

Table 15: Percent Total Ash (DM basis) Resulting from Combustion of Opuntia Samples Included in the Comparative Study.

Species	Mean
Alfalfa (ref. sp)	10.75
<i>O. engelmannii</i>	15.43
<i>O. filipendula</i>	19.60
<i>O. versicolor</i>	13.90
<i>O. polyacantha</i>	13.70
<i>O. fragilis</i>	11.58

The ashing of Opuntia samples resulted in relatively high ash residue (table 15) if compared to that of alfalfa samples analyzed for comparison and reference purposes. Explanation of this might be based on the high calcium, cellulose and hemicellulose proportions in Opuntia samples. Reduced volatile substances might be included in the assumptions, too.

5. ENERGY

The energy content of a feed, particularly its digestible and net portions, is of great importance to determine. All functions and biochemical processes of a living organism require energy. According to NRC (1984), the Digestible Energy (DE) attempts to measure the digestible energy (in weight units). The Metabolisable Energy (ME) estimates the remaining digested energy after

losses in urines and gases (chiefly methane). The Net Energy determines the actually available dietary energy for maintenance and production needs of the animal.

Table 16: Average Gross and Digestible Energy Content (kcal/kg, DM basis) of the analyzed Opuntia Species.

Species	Average GE(1)	S E		Average DE
Alfalfa (ref. sp)	4439.38	20.86		3024.55
<u>O. engelmannii</u>	3543.64	41.18	b	2098.54
<u>O. filipendula</u>	3288.09	14.11	c	1763.07
<u>O. versicolor</u>	3817.65	34.21	a	1927.15
<u>O. polyacantha</u>	3762.94	25.07	a	1687.30
<u>O. fragilis</u>	3895.08	16.41	a	1946.76

(1) Means followed by the same letter are not significantly different at the level 1%.

Gross energy determination led to the conclusion that Opuntia pads encompass a fairly high amount of energy. Combusted O. fragilis samples gave 3895.08 kcal/kg which was only 20% lower than that of alfalfa samples (4439.38 kcal/kg). Digestible Energy (DE) as calculated following Fulgham (1978) estimate equation:

$$DE = \text{Gross Energy} \times \text{IVDMD} (\%)$$

appears to be quite important given the relatively high In Vitro Dry Matter Digestibility (IVDMD) of most Opuntias. In the case of O. fragilis (the highest in Gross Energy), DE = 1946.76 kcal/kg and in the case of O. engelmannii (the highest in IVDMD), DE = 2091.54 kcal/kg. The Digestible Energy requirements for domestic livestock, through

use of Metabolisable Energy (ME), could be estimated following the NRC (1984) equation:

$$DE = ME \times 1.22$$

Using this equation, a dry pregnant mature cow (middle third of pregnancy) requires 2.14 Mcal/kg of digestible energy. Thus, Opuntia species analyzed are relatively close to provide the required Digestible Energy for cattle nutrition. Shoop et al., (1977) found that O. polyacantha (prickly pear) contained 2.6 Mcal of digestible energy per kilogram, the same amount as alfalfa hay and higher than that of grama grasses (Bouteloua spp.) containing only 1.6 Mcal/kg. This energy level of Opuntias could make them a valuable component to include in livestock rations. Moreover, cacti are cheap and easy to grow besides being abundant in the zone of frequent droughts.

6. DIGESTIBILITY

DRY MATTER DIGESTIBILITY

In Vitro Dry Matter Digestibility

In order to reduce the variability due to the digestion environment, the In vitro and In vivo dry matter digestibility analyses were run during the same period. Rumen fluid for the Two-stage In vitro Dry Matter Digestibility (IVDMD) analysis was taken from one of the cows being used for the In vivo trials. Under these condi-

tions, it may be assumed that the microbial action would be similar for the two types of digestion. The results obtained from the IVDMD are presented in table 17.

Table 17: Average In Vitro Dry Matter Digestibility (% DM basis) of the Opuntia Species.

Species	Mean
Alfalfa (ref. sp)	68.13
Grass hay (ref.sp)	29.35
<u>O. engelmannii</u>	59.22
<u>O. fragilis</u>	49.98
<u>O. filipendula</u>	53.62
<u>O. versicolor</u>	50.48
<u>O. polyacantha</u>	44.84

The analysis of the above results (table 17) indicates a relatively high digestibility for Opuntias particularly O. engelmannii (59.22%) and O. filipendula (53.62%). Their IVDMD proportions were close to that of alfalfa. All the analyzed Opuntia samples showed a higher percent digestibility than the result obtained in the case of grass hay. Similar conclusions were reported by Shoop and his collaborators (1977).

Marten (1981) suggested that the Acid Detergent Fiber (ADF) could be used to estimate the feed digestibility (inversely correlated). In the work, the fiber analyses showed that O. versicolor and O. polyacantha samples had the highest ADF content (18.98% and 18.72%, respectively) and consequently were among the least digestible species.

Their respective IVDMD rates were 50.48 and 53.48%. Conversely, O. engelmannii was lowest in ADF (11.29%) and highest in dry matter degradability (59.22%).

As occurred during Neutral Detergent Fiber filtration, some difficulties were, again, encountered at the In vitro filtration stage. This was due to cactus mucilagenous character. The phenomenon was particularly detected in the beakers containing O. versicolor and O. polyacantha samples. The interference of the mucilage was less intense in the case of the IVDMD than in that of NDF filtration. This difference in intensity could be related to the filter pore size or to the reagents used in the two cases.

In Vivo Dry Matter Digestibility

During the In vivo digestibility trials the fistulated cows were fed a medium quality grass hay composed of Agropyron cristatum and Bromus spp. Grass hay was chosen because it is the feed most frequently combined with cactus when formulating livestock rations in Tunisia.

In conducting In vivo Dry Matter Digestibility studies using the nylon bag technique, (Van Dyne (1962), Mehrez and Orskov (1976), Hellen and Ellis (1977), Cummins et al., (1983), Stern and Satter (1984) and Nocek (1985) emphasized the importance of pore size, and sample and bag sizes. In this study a pore size of 53 microns was

chosen, 1 g of ground plant material and 17x9 cm bag size were, also, chosen in agreement with the literature cited above. The analyses results were as follow (table 18):

Table 18: Average In vivo Dry Matter Disappearance (% - DM basis) of the Opuntia Species Included in the Comparative Study.

Species	Average Disappearance	
	After 24 hours	After 48 hours
Alfalfa (ref.)	58.40	59.41
grass hay (ref.)	27.48	32.47
<u>O. engelmannii</u>	53.09	57.22
<u>O. filipendula</u>	52.24	55.39
<u>O. versicolor</u>	43.45	48.30
<u>O. polyacantha</u>	42.21	44.61
<u>O. fragilis</u>	43.15	47.47

In vivo Dry Matter Disappearance (In Vivo DMD) were determined after two incubation periods, 24 and 48 hours. For both periods O. engelmannii had the highest percent digestibility (53.09% and 57.22%, respectively for 24 and 48 periods), and O. polyacantha showed the lowest In vivo DMD (42.21 and 44.61). The results obtained indicate that Opuntias showed a relatively high DM digestibility when compared to that of grass and alfalfa included as references. All the cactus samples resulted in a higher digestibility rates than that of grass hay. O. engelmannii DM Disappearance (57.22%) was close to that of alfalfa samples (59.41%).

Differences in Dry Matter Digestibility among groups of bags incubated in different cows were detected in the results of the two incubation periods of 24 and 48 hours. Similar observations were reported by Van Dyne (1962) analysing mixed range forage and Solka floc, and Mehrez and Orskov (1976) using grass hay. However, Nocek (1985) found that no significant ($P < 0.05$) differences existed between cottonseed meal samples incubated, for 24 and 48 hours, in 4 rumen fistulated cows.

For a given cow, the Nylon Bag Dry Matter Digestibility analyses resulted in insignificant ($P < 0.05$) differences between levels of DM disappearance after the 24 and 48 hours incubation periods. The slow and extended digestibility process, in the cases of O. versicolor and O. fragilis, might be related to their high ADF (lignin and cellulose) content. Diverse conclusions have been drawn regarding the incubation period effects on the significance of DM disappearance rates. Mehrez and Orskov (1976), analysing rolled barley on rumen-fistulated sheep maintained on grass hay, indicated that DM digestibility increased with time of incubation up to 15 hours. The increases were small when the incubation time was extended to 18 hours. According to these researchers there was no significant disappearance at the end of the 24 hours incubation period. However, Nocek (1985) studying cottonseed meal digestibility in rumen-fistulated cows observed

that the rates of DM Degradability rates were significantly ($P < 0.05$) different at 24 and 48 hours incubation periods.

In Vivo versus In Vitro Dry Matter Digestibility

The In vivo Dry Matter Disappearance results were slightly lower than the proportions obtained with the IVDMD method. Similar results, concerning O. phaeantha, were reported by Shoop et al. (1977). The nylon bag DM degradability of 66.4% (after 48 hours) and the In vitro (63.8%) were not significantly different at 5% level of error. Also, Menson and his collaborators (1969), comparing the In vivo DMD and IVDMD of Coastal Bermudagrass hay, obtained a high correlation between the two techniques ($r = 0.92$). The authors indicated that the IVDMD of low quality bermudagrass hay was consistently higher than the nylon bag DMD. However the latter was slightly higher for a better quality.

As was indicated by Marten (1981), Acid Detergent Fiber could be used to estimate the feed digestibility (inversely correlated). The Opuntias analyses showed a low Acid Detergent Fiber residue along with a low lignin content. Both components, together or separately, usually estimate digestibility of feeds. The cactus characteristic, mentioned frequently in literature, of being readily digestible could be proposed as an explanation to the

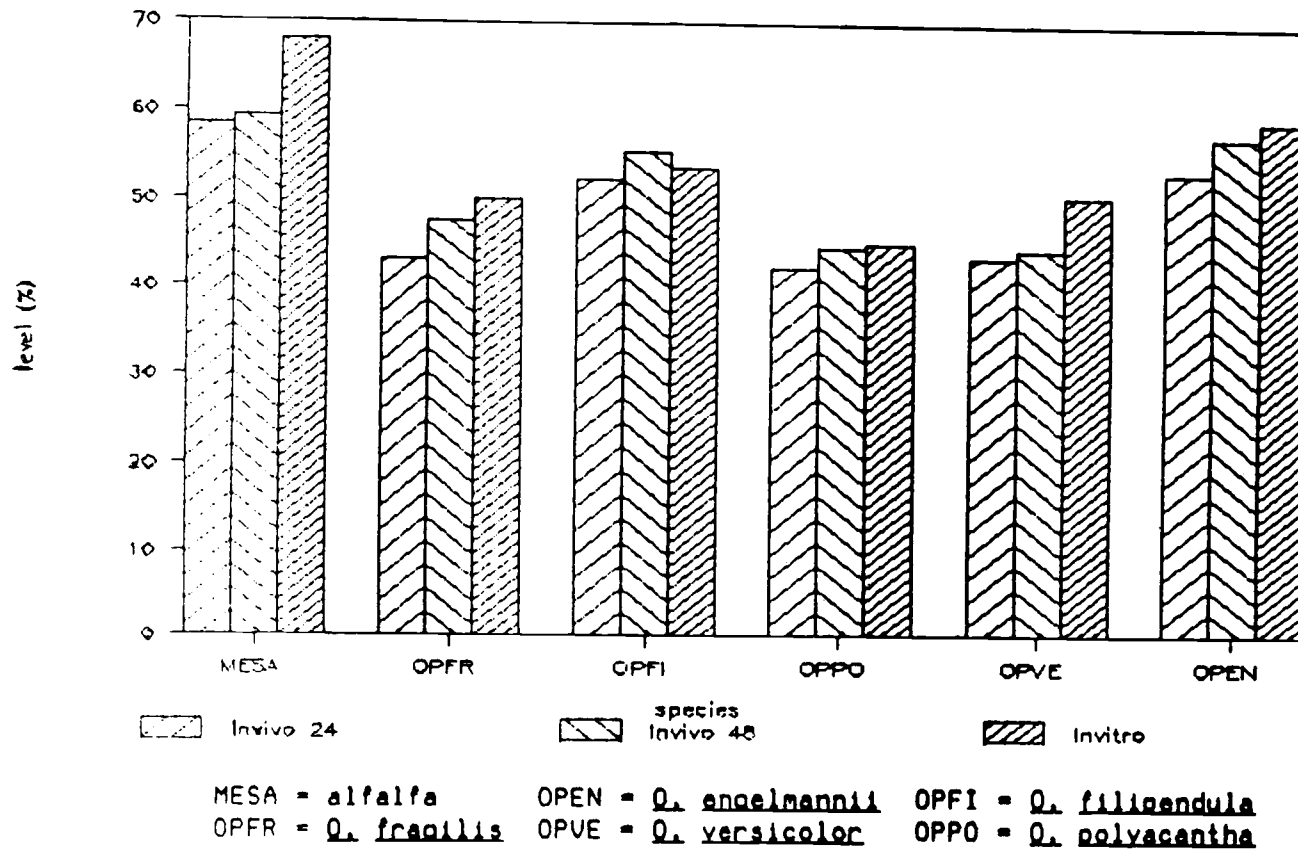


Figure 1. In vivo and In vitro dry matter digestibility of the Opuntia samples.

concentrated microbial activity during the first incubation period. The few available nutrients are degraded at the beginning of the first incubation period (of 24 hours). No significant nutrient extraction could be operated later in the incubation period. The analyses showed that O. versicolor and O. polyacantha had the highest ADF content (18.98% and 18.72%, respectively) and the lowest digestibility. The IVDMD analysis resulted in 50.48% and 44.84%, respectively for O. versicolor and O. polyacantha. Their respective rates as determined by the Nylon Bag technique were 48.30% and 44.61%. Conversely, the crude fiber analyses resulted in the lowest proportion of ADF in the case of O. engelmannii with a highest DM degradability (IVDMD 59.22% and In vivo DMD 57.22%).

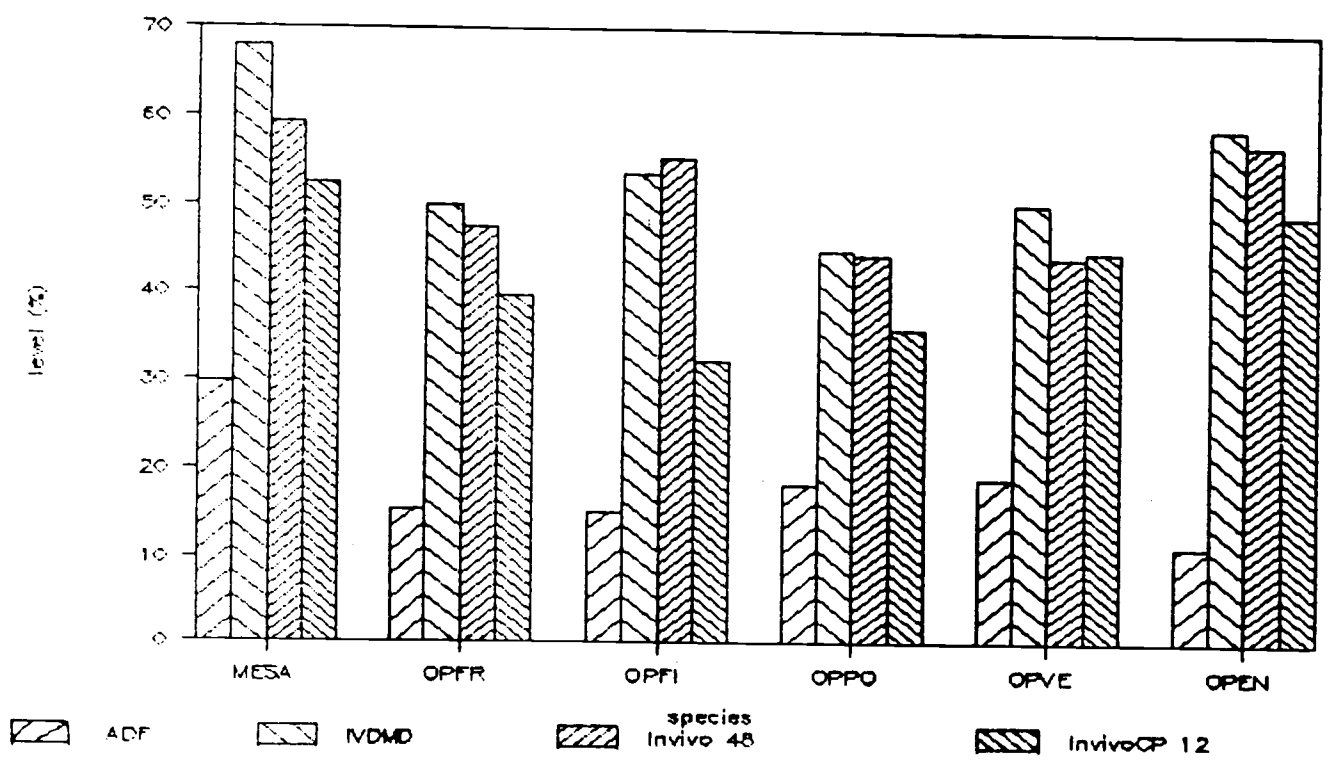
CRUDE PROTEIN DIGESTIBILITY

Table 19: Average In vivo Crude Protein Degradability (% , DM basis) of the Opuntia Species.

Species	Average Disappearance	
	After 6 hours	After 12 hours
Alfalfa (ref.)	52.54	59.49
grass hay (ref.)	33.12	36.90
<u>O. engelmannii</u>	49.20	51.80
<u>O. filipendula</u>	32.40	37.11
<u>O. versicolor</u>	45.00	46.81
<u>O. polyacantha</u>	36.06	36.61
<u>O. fragilis</u>	39.69	40.70

The Nylon Bag Crude Protein Disappearance after 6 hours and 12 hours incubation periods resulted in large differences between the Opuntias digestibility rates. More variability appeared to dominate data from the 6 hours-incubation period. O. engelmannii showed the highest crude protein degradability levels (49.20% and 51.80%, respectively for the 6 and 12 hours incubation periods). These rates were close to that of alfalfa (52.54% and 59.49%). The lowest nitrogen disappearance rates were obtained in the case of the O. filipendula and O. polyacantha samples. Losses in crude protein content of 32.40% and 36.06% were calculated respectively for O. filipendula and O. polyacantha after the 6 hours incubation period. The 12 hours period ended with disappearance rates of 37.11% and 36.61%, respectively for the two species. Compared to grass hay samples, all the Opuntia species showed higher crude protein digestibility.

From table 18 it appears that most of the digestion activities occurred during the first incubation period. These activities continued during the second period but slowly. Mehrez and Orskov (1976) studying In vivo CP digestibility of rolled barley using rumen fistulated sheep indicated that the nitrogen disappearance could be estimated from data obtained after 12 hours of incubation. Similar conclusions were reported by Nocek (1985) working on cotton seed meal.



MESA = alfalfa OPEN = *Q. engelmannii* OPFI = *Q. filipendula*
 OPFR = *Q. fragilis* OPVE = *Q. versicolor* OPPO = *Q. polyacantha*
 ADF = Acid Detergent Fiber IVDM = In vitro Dry Matter Digestibility
 In vivo CP = In vivo Crude Protein Digestibility

Figure 2. In vitro (two-stage) and In vivo (nylon bag technique) dry matter digestibility and In vivo crude protein digestibility (12 hours) as related to ADF content.

The effect of rumen microbial nitrogen on the crude protein degradation was not determined by this study but Nocek (1985) stated that no significance ($P < 0.05$) differences were detected between nitrogen disappearance rates with or without correction for microbial protein. The author indicated that the rate of contamination and/or attachment proceeded at a slower rate until 12 hours, and declined more rapidly after then. This researcher explained these characteristics through the limitation of attachment sites for continual digestion and/or substrate availability.

7. SUMMARY

All the Opuntias analyzed for this work were spiny. The density and size of spines were different among the species. Spines of O. fragilis pads were the longest and the more dense. Intending to study the Opuntia species in the conditions under which they are frequently consumed by cattle and camels, no treatments (singling off the spines) have been operated on the cactus samples before oven drying and grinding.

The analyses showed relatively low fiber content for the Opuntia samples when compared to that of grass hay and/or alfalfa hay. O. versicolor was the highest in all fiber components and O. engelmannii had the lowest proportions. As a consequence of this low fiber content,

Opuntias soluble portion resulting from Neutral Detergent solution action was higher than that species reference. Further analyses the components of this soluble portion are necessary to have an over-all view of the Opuntias nutritional value. The crude fiber proportion of all the species was very low. O. versicolor, was the highest (6.83%) and O. filipendula was the lowest (2.95%). In vivo crude protein digestibility, through the use of nylon bag techniques, showed that less than the half of this protein content was available to the rumen microbes. The other portion could be under ligno-proteic complex. In arid and semi arid region, where cactus is a basic component of livestock feeding particularly during periods of drought, the supplementatation with a protein source is necessary.

The phosphorus content of Opuntias samples was low and, in most of the cases less than livestock requirements. O. fragilis was the highest in and O. versicolor had the lowest phosphorus concentration. With regard to calcium levels, the analyses showed that Opuntias have very high concentrations. O. filipendula samples, the highest in calcium content, contained about 5 folds the calcium proportion of alfalfa samples. Among the Opuntia species, calcium-phosphorus ratio (Ca/P) varied from 30 to 77. This nonequilibrated mineral (Ca and P) content of Opuntias enables livestock to reach their optimum

performance. In order to correct the phosphorus deficiency of cactus a mineral supplement particularly high in phosphorus is needed.

Gross energy determination led to the conclusion that Opuntia pads encompass a fairly high amount of energy. O. fragilis, the highest in Gross Energy, has an estimated Digestible Energy of 1.85 Mcal/kg and O. engelmannii, the highest in In Vitro Dry Matter Digestibility, measured a Digestible Energy of 2.02 Mcal/kg.

Opuntias Dry Matter Digestibility rates were relatively high when compared to the species included as reference. This was mainly due to their low fiber content. However, these rates were lower than expected because of the very low protein content of cactus.

Finally, it could be concluded that, the difference in nutrients content between species were significant. However, no clear separation appeared between groups of species. Also, there was no one or more species that consistently maintained the highest (or lowest) results through all analyses.

- O. engelmannii was lowest in fiber content and, consequently, had the highest Dry Matter and Crude Protein digestibility (In vitro and In Vivo).

- O. versicolor (the unique cylindropuntia included in the study) had the highest crude fiber and crude protein proportions and was the second highest at the gross

Table 20. Recapitulative Table of
Major Opuntia Samples
Nutrients and Digestibility
Rates (% , DM basis).

	Alfalfa Hay	Grass Hay	Opuntia engelm	Opuntia filipen.	Opuntia versicol.	Opuntia polyacan.	Opuntia fragil.
C. FIBER							
NDF	45.15	—	31.18	33.30	39.85	31.16	35.08
ADF	29.91	—	11.29	15.31	18.98	18.42	15.47
Hemic.	15.24	—	19.88	17.99	20.87	12.74	19.61
Cellu.	21.48	—	7.95	10.49	13.73	12.69	10.37
Lignin	7.93	—	2.89	3.97	3.86	4.79	3.91
C PROTEIN	18.29	8.84	4.16	2.95	6.83	3.61	3.44
PHOS (P)*	0.19	—	0.16	0.17	0.08	0.16	0.29
Calcium *	1.68	—	5.79	9.71	6.21	6.77	6.33
Total Ash	10.75	—	15.43	19.60	13.90	13.70	11.58
Gr. Ener.	4439.38	—	3543.64	3288.09	3817.65	3762.94	3895.08
Dig. Ene.	3024.55	—	2098.54	1763.07	1927.15	1687.30	1946.76
IVDMD	68.13	29.35	59.22	49.98	53.62	50.48	44.48
<u>In vivo</u> DM1	58.40	27.48	53.09	52.24	43.45	42.21	43.15
<u>In vivo</u> DM2	59.41	32.47	57.22	55.39	48.30	44.61	47.47
<u>In vivo</u> CP3	52.54	33.12	49.20	32.40	45.00	36.06	39.69
<u>In vivo</u> CP4	59.49	36.90	51.80	37.11	46.81	36.61	40.70

- Gr. Ener. = Gross Energy (kcal/kg)
- Dig. Ene. = Digestible Energy (kcal/kg)
- IVDMD = In vitro two-stage Dry Matter Digestibility
- In vivoDM1 = In vivo Dry Matter Digestibility after 24 hrs.
- In vivoDM2 = In vivo Dry Matter Digestibility after 48 hrs.
- In vivoCP3 = In vivo crude protein digestibility after 6 hrs.
- In vivoCP4 = In vivo crude protein digestibility after 12 hrs.

energy level but was the lowest in Dry Matter and Crude Protein digestibility besides phosphorus concentration.

- O. polyacantha was among the group having highest fiber content and second lowest DM and CP disappearance. It had the second highest calcium proportion.

- O. filipendula was among the group of highest fiber content, the highest in total ash and calcium concentrations.

- O. fragilis had the highest gross energy and phosphorus contents.

In conclusion, Opuntia species appear to be relatively low crude fiber content with considerably high Dry Matter and Crude Protein digestibility rates. Also, most Opuntias had appreciable gross and digestible energy levels. This added to their high moisture content, abundance in arid and semi arid regions (without cultivation), easy and cheap to grow (if needed to be cultivated), high resistance to droughts. All of these factors make the Opuntias an appreciable feed to include in livestock rations with attention given to their low and incompletely available crude protein besides the low phosphorus levels. As was clearly stated in literature, livestock should not be maintained on cactus only, but this type of feed could fit easily in a ration based on grasses. Rations could be based on Opuntias but, as stated above, a protein and mineral source should be provided.

SECTION II: NUTRIENT SEASONAL VARIABILITY

Opuntia fragilis was analyzed for seasonal variability of its major components. The samples were collected from the John Day region in Central Oregon, on February 7th, May 15th, August 18th and November 5th of 1986. The vegetative material was collected from several plants, dried and ground as was described in Vegetative Material and Experimental Methods chapter. The samples were analyzed for Neutral Detergent Fiber, Acid Detergent Fiber, hemicellulose, cellulose, lignin, crude protein, calcium, phosphorus, and gross energy content, in addition to In vitro Dry Matter Digestibility tests. Winter, spring and summer samples, along with that of alfalfa, were analyzed in quadruplicate for fiber components and in duplicate for the other nutrients. Due to the late collection of Fall samples, they were not analyzed for dry matter digestibility. For comparison and for verification of results, samples from winter, spring and summer along with that of alfalfa, were rerun with the Fall samples. The results found for these reanalyzed samples were very close to those obtained previously. Thus, the preceding results from winter, spring and summer analyses were used in the comparisons.

1. COLLECTION SITE DESCRIPTION

The samples were collected from a west facing slope near the John Day Fossil Beds National Monument, Central Oregon. John Day zone climatological data, of 33 years, was provided by the Climatic Research Institute - Oregon State University, in November 1986 (table B1). According to this source, annual precipitation averaged 12.432 inches and was primarily received in winter and spring. The lowest temperatures were reached in winter (January has the lowest average: 20.8 degrees F). Summer is the warmest season with July having the highest average temperature (88.4 degrees F). According to a USDA report (Evenden, 1983), soils are stony primarily a clay loam belonging to the Simas series.

Table 21: Monthly Average Precipitation, Maximum, Minimum and Mean Temperatures of the John Day Fossil Beds region (33 years).

Months	Average Precip. (inches)	Average Maximum Temp. (F)	Average Minimum Temp. (F)	Average Temp. (F)
January	1.22	40.1	20.8	30.4
February	0.82	46.8	25.3	36.1
March	1.09	52.1	27.8	40.0
April	1.21	59.2	32.1	45.6
May	1.66	68.7	38.5	53.6
June	1.45	77.6	44.7	61.2
July	0.84	88.4	48.6	68.5
August	0.90	87.0	47.3	67.2
September	0.85	77.8	40.5	59.2
October	1.03	65.4	33.4	49.4
November	1.36	50.3	27.8	39.1
December	1.81	42.7	23.0	32.6

2. CRUDE FIBERTable 22: Crude Fiber Seasonal Variability in O. fragilis (% , Dry Matter Basis).

Component	Alfalfa (ref.)	<u>Opuntia</u>			
		Seasons			
		spring	summer	fall	winter
Neutral Detergent Fiber	47.14	36.23	38.10	37.97	35.19
Acid Detergent Fiber	31.33	17.26	19.37	18.96	17.51
Hemicellulose	15.81	18.97	18.73	19.01	17.68
Cellulose	19.78	12.18	12.31	10.02	11.16
Lignin	7.10	4.08	6.52	8.13	5.61

Generally speaking, the results in table 22 indicate that crude fiber content in Fall samples was close to that found for summer collection. Also, this table shows that fiber content increased from spring to summer, then stabilized, in level, through the fall. Winter collection resulted in slightly lower fiber proportions than that of Fall samples. Crude fiber content, as well as the other cactus plant nutrients, is closely related to the plant growth process and growing season patterns. Dart (1981) indicated that the pattern of growing season in Oregon is longest along the coast and becomes shorter as the distance in land and the local elevation increase. According to this author, the interior valleys of Central and Eastern Oregon have a growing season that ranges from 50 to

150 days depending on the elevation and topography. The author also indicated that these regions are characterized by an early frost and late spring. Under the described climatic conditions, plant (cactus) growth starts late in the spring. The newly formed vegetative material, as a result of growth, contains significantly lower fiber components than the old cactus pads. Since Spring samples were collected during the second half of the season (May 15th) it would include some of the young pads. Consequently the fiber analyses resulted in low components content for spring samples. As was stated in a USDA report (Evenden, 1983) the John Day region receives its most important rainfall fraction during winter and spring. Due to this available moisture and warm weather, cactus synthesizing activities continued in summer. Thus, some more fiber was formed during this season in addition to the quantity resulting from the 'lignification', of vegetative material already synthesized in spring. Consequently, the analyses showed a higher fiber content in summer samples than that of spring. Fall results indicate that fiber components maintained, almost, the same content as in summer samples with a slight increase in lignin. This increase in lignin proportion indicates that spring growth reached maturity and no more young pad formation had occurred during late summer and fall. The almost stabilization of fiber components from summer to

fall was followed by a slight decline during the winter. This could be related to the cactus plant physiological activity which was more oriented toward synthesis of other nutrients, such as soluble carbohydrates and minerals, rather than fiber components. For example phosphorus levels increased from fall to winter (table 24) and crude protein content was higher in Fall samples than in that of summer (table 25).

3. CRUDE PROTEIN

Protein is the component of highest concentration in animal tissue. All cells synthesize protein for part or all of their functioning cycles, and without protein synthesis life could not exist (Church and Pond, 1978).

Table 23: Crude Protein Seasonal Variability in O. fragilis (% , Dry Matter Basis).

Component	Alfalfa	<u>Opuntia</u>			
	(ref.)	spring	summer	fall	winter
C. Protein	18.26	3.73	3.55	3.59	3.24

As was indicated in section I, crude protein levels in O. fragilis like other Opuntias, were very low. The same conclusion was drawn from the seasonal analyses in which crude protein maintained an almost constant level through spring, summer and fall. Winter collection resulted in

the lowest protein proportions. This could be due to a decrease in nitrogen absorption by the cactus plant resulting from losses of nitrogenous compounds through erosion or percolation. In a USDA soil survey of Prineville region (1966) it was indicated that the churning of Day series soil type tends to move organic material downward, and subsoil material towards the surface.

4. MINERALS: PHOSPHORUS AND CALCIUM

All animals require minerals in certain amounts both for maintenance and production purposes. They are needed in certain quantities and must also be available in a certain ratio to each other (Yates, 1985). Two of the most important minerals required in livestock rations are phosphorus and calcium. According to NRC (1984) the ratio of these two minerals, in cattle diets, should never be higher than 7 parts calcium to 1 part phosphorus.

Table 24: Seasonal Variability of Phosphorus and Calcium Content in O. fragilis (% , Dry Matter Basis).

Component	Alfalfa (ref.)	<u>Opuntia</u>			
		spring	summer	fall	winter
Phosphorus	0.23	0.27	0.30	0.25	0.29
Calcium	1.47	6.28	6.53	6.31	6.10

As was found in section I, in mineral analyses, O. fragilis has a high calcium concentration and a phosphorus content within the range of livestock requirements. The phosphorus proportions maintained an almost constant level through the seasons. As appears from table 24, calcium levels increased from spring through summer (highest proportions). This increase seems to follow a similar trend as fiber components. It might be suggested that during late spring, summer and early fall, Opuntia synthetical activities were oriented more toward structural matter. After reaching maturity these activities were concentrated on the synthesis of cell soluble nutrients such as carbohydrates.

5. ENERGY

Energy is, quattitatively, the most important item in an animal diet, and all animal feeding standards are based on energy needs (Church and Pond, 1978).

Table 25: Seasonal Variability of Gross and Digestible Energy (kcal/kg, Dry Matter Basis) in O. fragilis.

Energy	Alfalfa (ref.)	<u>Opuntia</u>			
		spring	summer	fall	winter
Gross Energy	4392.6	3895.8	3800.9	3690.7	3682.0
Digestible Energy	2992.7	1922.3	1655.7	(1)	1737.9

(1) Digestible Energy was not estimated (see text).

Digestible Energy (DE) was estimated using the equation suggested by Fulgham (1978):

$$DE = \text{Gross Energy} \times \text{IVDMD} (\%)$$

Since the variability in dry matter digestibility (IVDMD) was not very high through the seasons, the Digestible Energy estimate followed almost the same trend of variation as digestibility: Spring and winter had close levels (slightly higher in spring), summer samples had the lowest Digestible Energy. Digestible Energy of Fall sample was not estimated because of unavailability of dry matter digestibility rates for these samples (late collection).

Warm and moist spring induced cactus growth. The plant deposited considerable amounts of nutrients in their joints and consequently, the stored Digestible Energy in these nutrients was relatively high. The young pads were highly digestible thus, increased the Digestible Energy levels in Spring samples.

6. IN VITRO DRY MATTER DIGESTIBILITY

Digestibility evaluation of feeds is very helpful in estimating their nutritive value and is necessary to formulate adequate rations for livestock feeding. The digestibility rates of a plant vary with its age, the season, and the way of forage use (fresh or air-dried).

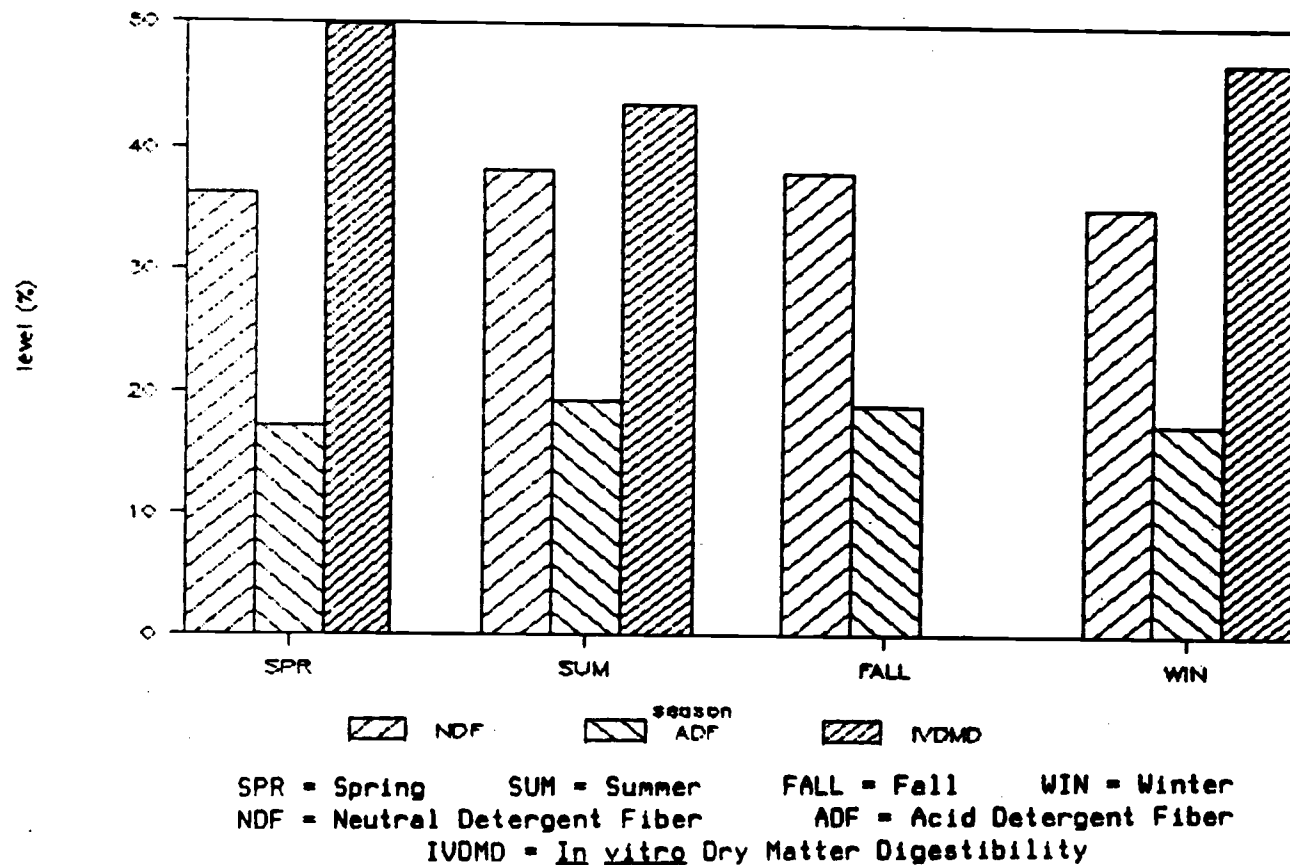


Figure 3. Seasonal variability of In vitro dry matter digestibility as related to Acid Detergent Fiber content (%) in Opuntia fragilis.

Table 26: In Vitro Dry Matter Digestibility (%) of O. fragilis Following the Seasons.

	Alfalfa (ref.)	Opuntia			
		spring	summer	fall (1)	winter
IVDMD (%)	68.13	49.98	43.56	—	47.20

(1) Fall samples were not analyzed for In Vitro Dry Matter Digestibility (IVDMD) variability with seasons because of late collection.

From the results presented in table B6 and those of table B2, it appears that the In Vitro Dry Matter Digestibility (IVDMD) rates varied inversely to the crude fiber levels (Neutral and Acid Detergent Fiber residues). This seems to be logical since the most important factor determining dry matter degradation is crude fiber, particularly lignin and cellulose. Marten (1981) indicated that Acid Detergent Fiber content of forages determine their digestibility rates. Digestibility of spring cactus samples was highest. This may be due to limited lignification of young pads. In summer, young pads became older, more structural fiber (cellulose and lignin) was deposited in their cells thus reduced the dry matter digestibility. The same proposal could be applicable to fall and winter samples.

Even though crude fiber content was low, the dry matter digestibility of O. fragilis samples (for all

seasons) was lower than expected. This could be due to the mucilaginous character of cactus that rendered dry matter degradation difficult to salivary enzymes and rumen microbes (brought through the inoculum).

7. SUMMARY

O. fragilis was analyzed for seasonal variability in its major components. Four pad samples were collected, through the year, from the John Day Fossil Beds region. These samples were analyzed for Neutral Detergent Fiber, Acid Detergent Fiber, hemicellulose, cellulose, lignin, crude protein, phosphorus, calcium and gross energy content, in addition to In Vitro Dry Matter Digestibility (IVDMD). The following table (27) summarizes the content of these nutrients and their seasonal variability.

An increase in the proportions of the crude fiber components was observed from spring to summer followed by an almost stabilization from summer to fall then a slight decline in winter samples. Calcium levels followed the same trend but the differences were only significant at the 5% level. With regard to phosphorus concentration, no significant variations were detected through the seasons. Crude protein content was highest in spring then declined slightly through out the seasons. The In Vitro Dry Matter Digestibility rates were almost equal in spring and winter samples and lower in the case of summer collection. Fall

Table 27: Recapitulation of major nutrients in O. fragilis and their Seasonal variability (% , Dry Matter Basis).

Component	Alfalfa (ref.)	Opuntia			
		spring	summer	fall	winter
Neutral Detergent Fiber	47.14	36.23	38.10	37.97	35.19
Acid Detergent Fiber	31.33	17.26	19.37	18.96	17.51
Hemicellulose	15.81	18.97	18.73	19.01	17.68
Cellulose	19.78	12.18	12.31	10.02	11.16
Lignin	7.10	4.08	6.52	8.13	5.61
Crude protein	18.26	3.73	3.55	3.59	3.24
Phosphorus	0.23	0.27	0.30	0.25	0.29
Calcium	1.47	6.28	6.53	6.31	6.10
Gross Energy	4392.6	3895.8	3800.9	3690.7	3682.0
Digestible Energy	2992.7	1922.3	1655.7	-	1737.9
<u>In vitro</u> Dry Matter Digest.	68.13	49.98	43.56	-	47.20

samples were not analyzed for dry matter degradation because of unavailable time (late collection).

This seasonal variability in Opuntia fragilis components is related to plant synthetical processes as influenced by the growing season patterns. Rapid growth in spring resulting in less lignified thus highly digestible plant material. Because spring samples included certain proportions of these young and highly digestible pads,

their energy content was higher than that of other collections. Fiber content stabilized from summer to fall primarily because spring growth reached maturity and the cells had completed the synthesis of their structural material (fiber).

The differences in all components through the seasons were small. A part of this variation was due the sampling procedure. Thus, this variability should not be given too much importance when planning to feed Opuntia fragilis. Cactus pads could fed to livestock during any season. Attention should be given to their high Calcium/Phosphorus ratio. The combination of Opuntia fragilis with low calcium or high phosphorus feeds is beneficial to allow optimum livestock performance. Opuntia fragilis low crude protein content should be corrected through supplementation of a protein source.

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APPENDICES

APPENDIX A

Crude Fiber as Determined by the Goering and Van Soest Procedure
 Described in the Agricultural Handbook No. 379.
 Size of the sample is .35 g.

SPECIES	NDF(CWC) (%)	Mean (%)	ADF (%)	Mean (%)	Hemicel (%)	Mean (%)
ALFALFA	46.00 45.32 44.45 44.82	45.15	29.59 30.30 30.07 29.66	29.91	16.41 15.02 14.38 15.16	15.24
<u>O. ENGELM.</u>	33.05 29.54 31.68 30.45	31.18	11.13 11.03 12.20 10.82	11.30	21.92 18.51 19.48 19.63	19.88
<u>O. FILIPE.</u>	32.29 31.37 33.96 35.59	33.30	14.23 14.68 16.93 15.41	15.31	18.06 16.69 17.03 20.18	17.99
<u>O. VERSIC.</u>	42.47 38.41 39.80 38.71	39.85	19.82 19.14 18.96 17.99	18.98	22.65 19.27 20.84 20.72	20.87
<u>O. POLYAC.</u>	31.03 29.40 30.47 33.73	31.16	18.27 19.06 18.04 18.31	18.42	12.76 10.34 12.43 15.42	12.74
<u>O. FRAGIL.</u>	34.16 32.50 35.02 38.63	35.08	14.73 12.79 15.84 18.52	15.47	19.43 19.71 19.18 20.11	19.61

Crude Fiber (Continued)

SPECIES	Lignin (%)	Mean (%)	Cellu (%)	Mean (%)	A I Ash (%)	Mean (%)
ALFALFA	7.98 8.74 7.55 7.46	7.93	22.21 20.04 21.93 21.76	21.48	0.40 0.54 0.60 0.45	0.50
<u>O. ENGELM.</u>	2.76 2.73 3.16 2.93	2.89	7.07 7.40 8.35 8.97	7.95	0.93 0.89 0.80 0.82	0.86
<u>O. FILIPE.</u>	4.05 3.98 4.14 3.65	3.96	9.35 10.97 11.10 10.54	10.49	0.77 0.63 0.72 0.82	0.73
<u>O. VERSIC.</u>	4.55 3.46 3.58 3.85	3.86	14.24 12.59 14.31 13.78	13.73	0.03 0.09 0.06 0.07	0.06
<u>O. POLYAC.</u>	4.69 4.72 4.84 4.91	4.79	11.34 12.02 12.98 14.42	12.69	0.53 0.51 0.62 0.58	0.56
<u>O. FRAGIL.</u>	4.39 3.92 3.69 3.62	3.91	8.98 11.93 9.75 10.84	10.37	0.24 0.26 0.21 0.30	0.25

APPENDIX B

Crude Protein as Analyzed Following the
Copper Catalyst Kjeldahl Micro Method.
Size of the Sample is 1 g.

Species	% Crude Protein	% CP (mean by species)
Alfalfa	17.98 18.59	18.28
Grass Hay	8.83 8.84	8.83
<u>O. engelm.</u>	4.07 4.24	4.16
<u>O. versicolor</u>	6.49 7.17	6.83
<u>O. Polyacantha</u>	3.87 3.35	3.61
<u>O. filipendula</u>	3.00 2.89	2.95
<u>O. fragilis</u>		
Spring	3.53 3.35	3.44
Summer	4.79 4.45	4.62
Fall	3.24 3.94	3.59
Winter	3.30 3.36	3.33

APPENDIX C

Dry Matter Content Determined on Oven Dried Samples
 at 100°C, Followed by An Ashing at 500°C
 to Determine the Total Ash Content on 1 g. Samples.

Species	DRY MATTER		TOTAL ASH	
	In Samples	Mean (species)	In Samples	Mean (species)
Alfalfa	95.43 95.41	95.42	10.72 10.77	10.74
<u>O. fragilis</u>	95.39 95.54	95.47	11.46 11.70	11.58
<u>O. engelm.</u>	95.68 95.68	95.68	15.37 15.49	15.43
<u>O. versicolor</u>	95.68 95.23	95.46	13.94 13.86	13.90
<u>O. polyacantha</u>	95.10 95.51	95.31	13.81 13.58	13.70
<u>O. filipendula</u>	92.71 93.08	92.90	19.30 19.90	19.60

APPENDIX D

Phosphorus Content Determined Following the Vanadomolybdate Procedure Using a Regular Spectrophotometer and Calcium Portion Using the Atomic Absorption Spectroscopy. The size of the sample is 1 g.

Species	PHOSPHORUS		CALCIUM	
	Samples (%)	Average (%)	Samples (%)	Average (%)
Alfalfa	0.19 0.19	0.19	1.76 1.59	1.67
<u>O. engelmannii</u>	0.17 0.14	0.16	5.11 6.47	5.79
<u>O. versicolor</u>	0.09 0.07	0.08	6.96 5.47	6.21
<u>O. polyacantha</u>	0.15 0.18	0.17	7.75 5.79	6.77
<u>O. filipendula</u>	0.19 0.14	0.16	10.68 8.75	9.71
<u>O. fragilis</u>				
Spring	0.22 0.17	0.20	6.22 6.45	6.34
Summer	0.24 0.21	0.23	5.20 4.76	4.98
Fall	0.26 0.24	0.25	6.12 6.49	6.31
Winter	0.22 0.20	0.21	5.51 4.35	4.93

APPENDIX E

Gross Energy Determined Using the Bomb Calorimeter
on 1 g Samples, and Digestible Energy Estimated
Using the In Vitro Dry Matter Digestibility Data.

Species	Gross Energy (kcal/kg)	
	Sample	Average
Alfalfa	4480.2978 4411.8970 4425.9439	4439.3796
<u>O. filpendula</u>	3304.5209 3260.0058 3299.7449	3304.5209
<u>O. versicolor</u>	3790.1848 3777.1075 3885.6548	3790.1848
<u>O. polyacantha</u>	3670.3705 3911.5896 3706.8703	3670.3705
<u>O. engelmannii</u>	3500.2676 3504.6977 3625.9652	3500.2676
<u>O. fragilis</u>		
Spring	3871.0513 3887.7387 3926.4404	3895.0768
Summer	3838.3002 3832.2948 3732.0957	3800.8969
Fall	3657.4996 3712.4468 3702.1123	3690.6862
Winter	3703.4042 3686.2241 3659.0061	3682.8781

APPENDIX F

The Two-stage In Vitro Dry Matter Digestibility
 Using the Procedure of Tilly and Terry
 as Modified by Alexander and McGowan.
 The size of the sample is 1 g.

Blk Res. = 0.070034 g
 Filt. DM = 97.76 %

Species	<u>In Vitro</u> DM Digestibility	
	Samples	Average
Alfalfa	65.82 69.53 69.04	68.13
Grass hay	30.90 28.07 29.09	29.35
<u>O. engelmannii</u>	59.35 60.89 57.43	59.22
<u>O. versicolor</u>	52.45 48.08 52.00	50.84
<u>O. filipendula</u>	54.87 51.70 54.30	53.62
<u>O. polyacantha</u>	46.52 43.41 44.58	44.84
<u>O. fragilis</u>	45.48	45.33
winter	46.85 43.66	
spring	51.50 49.69 48.75	49.98
summer	54.85 50.36 53.17	52.80

APPENDIX G

In Vivo Dry Matter Digestibility
 Determined on Five Rumen Fistulated Steers
 Fed Grass Hay.
 The size of the sample is 5 g.

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<u>In Vivo</u> Dry Matter Digestibility						
Species	Sample	Average	Species	Sample	Average	
ALFALFA	after 48 h	59.40	<u>O. POLYACAN.</u>	43.53	44.61	
				61.35		45.33
				18		47.55
				58.44		45.99
				58.58		40.66
	after 24 h	58.39		42.21	45.20	
					56.43	41.46
					57.85	40.87
					59.62	43.64
					59.25	39.88
GRASS HAY	after 48h	32.47	<u>O. VERSICOLOR</u>	46.40	48.30	
				32.12		49.76
				31.06		51.43
				33.27		48.32
				32.66		45.57
	after 24h	27.48		43.45	46.40	
					26.54	45.33
					29.54	43.22
					27.54	41.33
					25.03	40.99
<u>O. ENGELM.</u>	after 48h	57.22	<u>O. FILIPENDULA</u>	55.47	55.39	
				56.83		50.44
				59.43		57.85
				57.63		58.85
				53.78		54.33
	after 24h	53.09		52.23	50.99	
					53.71	53.29
					50.75	

In Vivo Dry Matter
Digestibility
(Continued)

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<u>In Vivo</u> Dry Matter Digestibility		
Species	Sample	Average

<u>O. FRAGILIS</u>		
Sp.	after 48h	45.99
		47.70
		48.10
		49.57
		45.99
S	after 24h	41.75
		43.76
		40.82
		44.10
W	after 48h	44.77
		43.54
		41.86
		49.47
		47.35
W	after 24h	30.58
		32.32
		35.29
		38.51
		39.23
Su	48h	36.17
		39.65
		37.71
		41.11
		43.65
Su	after 24h	33.58
		35.65
		32.79
		37.32
		38.12

APPENDIX H

In Vivo Crude Protein Disappearance
Using 3 Rumen Fistulated Steers Fed Grass Hay.
Size of the sample is 5 g.

Species	CP Digesti- bility (%)	% CP Digesti. (mean by Species)	Species	CP Digesti- bility (%)	% CP Digesti. (mean by Species)
Alfalfa after 6 h	55.76 49.04 52.82	52.54	<u>O. filpendula</u> after 6 h	33.64 30.1 33.45	32.40
after 12 h	59.61 56.32 62.54	59.49	after 12 h	36.82 39.03 35.47	37.11
Grass Hay after 6 h	35.03 31.63 32.7	33.12	<u>O. polyacantha</u> after 6 h	34.89 37.51 35.78	36.06
after 12 h	39.05 37.01 34.64	36.90	after 12 h	38.52 35.22 36.08	36.61
<u>O. fragilis</u> after 6 h	42.81 39.03 37.22	39.69	<u>O. versicolor</u> after 6 h	47.63 44.38 42.99	45.00
after 12 h	43.74 38.01 40.36	40.70	after 12 h	45.86 48.25 46.31	46.81
			<u>O. engelmannii</u> after 6 h	46.84 51.91 48.84	49.20
			after 12 h	54.84 49.53 51.04	51.80