ESTIMATION OF THE PROPORTIONS OF GRASS AND LEGUME IN EXTRUSA OF ESOPHAGEALLY-FISTULATED ANIMALS

by

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Chapter I

INTRODUCTION

The botanical and chemical composition of the grazing animal's diet is directly related to animal performance and production. A better knowledge of plant parts and quality of the forages ingested by the animal is needed in order to utilize more efficiently these valuable nutritional resources. However, many of the methods used to determine the forage ingested are not fully reliable.

In the past 30 years the esophageal-fistula technique has been widely used to study the ingested forage and the behaviour of the grazing animal. Various methods have been developed to determine the botanical composition of the forage grazed by fistulated animals. The disadvantages of these visual techniques are the low accuracy and the great amount of time required for each analysis. Mastication and salivation also affect the physical structure of the forage samples. The constituent differential method developed by Cooper et al. (1957) is an alternative method instead of the subjective visual methods. This method is based on the chemical characteristics of the species being grazed and therefore is more precise.

This research was undertaken to evaluate the constituent differential method in the estimation of grass and legume proportions in extrusa samples from esophageally-fistulated animals. The reliabilty of the method was examined in different known grass:legume combinations and in mixtures fed to fistulated steers. The constituents studied were neutral detergent fiber (NDF), calcium (Ca) and lignin.

Chapter II

LITERATURE REVIEW

The techniques commonly used to examine the diet of the grazing animal may be grouped into indirect methods and diet sampling techniques.

Direct Observation of the Animal

One of the oldest and most commonly used techniques is the simple observation of the grazing animal (Theurer, 1970). The advantages of this method are the simplicity, minor equipment requirements and the possibility of use in any season or situation (Bjugstad et al.1970). The major drawbacks are the subjectivity of the results, particularly the identification of species and the quantification of the amounts consumed (Minson et al., 1976).

Other methods used to aid in the quantification of the botanical composition by direct observation of the animal are the bite-count method and the time-spent-feeding method. In either case the proportions of each species in the diets are related to the number of bites or the number of minutes of feeding (Reppert, 1960).

Utilization Estimates

Forage utilization has been defined as the degree to which animals remove the current growth of herbage, expressed as a percentage of growth within reach of the grazing animal (Soc. Amer. Foresters, 1950). Limitations of such methods include large losses of plants and plant parts from weathering, trampling and consumption by animals other than those under study (Cook and Stoddart, 1953). Regrowth after defoliation can reduce the accuracy of utilization estimates (Holechek et al., 1982).

Ocular Estimate-by-Plot. Ocular estimates are obtained rapidly in the field and computation time is fast, but operators require considerable training (Smith et al., 1962). The estimates are made on small plots by one or more operators. The plots are clipped, simulating grazing, and estimates of the forage removal are made. The stubble is clipped and the actual difference in weight is computed. An investigator tests the accuracy of estimates each day by clipping and weighing herbage on at least 10 plots (NAS-NRC, 1962).

Studies comparing ocular estimates and esophageal fistula samples showed similar results when analyzing botanical composition and utilization (Laycock et al., 1972; McInnis et al., 1983). These researchers showed that ocular estimates resulted in diets lower in graminoids and higher in forbs

than other methods. Laycock et al. (1972) observed 'invisible utilization' of forbs, which were pulled from the ground by sheep.

Caged Plots. The caged plot methods involve the comparison of the amount of herbage present inside a cage with that outside (Holechek et al., 1982). A series of pairs of quadrats are located in a pasture prior to grazing. All quadrats are marked and one of each pair is randomly selected to be caged. Immediately after grazing the quadrats are clipped to ground level. The amount and percentage of each species removed from the open quadrats are calculated as the difference between herbage weight in caged quadrats and that in open quadrats (Laycock et al., 1972).

Size of quadrats may vary between 30 cm^2 to several square meters although smaller plots are more desirable (NAS-NRC, 1962). Various types of portable cages have been used in different situations (Fenley, 1951; Brown, 1954; Prendergast and Brady, 1955). The principle difficulty of the method is the change in microclimate inside the exclosures (Owensby, 1969). Cowlishaw (1951) reported that yields in caged quadrats were greater than in open quadrats. He attributed the greater growth to the reduction in wind velocity and the increase of humidity under the cages. These changes may be reduced with the use of open-mesh wire cages (Heady, 1957).

Studies comparing three methods of diet utilization showed that the caged plot method was the best way to measure the total impact of grazing (Laycock et al., 1972). However, results from this method were less consistent with data from esophageal fistulation than with data from the ocular estimate-by-plot method.

Methods Based on Mathematical Models. These methods are based on measurements, correlations and regressions of factors related to utilization. The methods include: the reduction in height method (Pechanec and Pickford, 1937); the stubble-height-class method (Canfield, 1944a); the height-weight ratio method (Lommasson and Jensen, 1943); the stem-count method (Stoddart, 1935); the short-cut method (Canfield, 1944b); the percent of plants ungrazed or grazed (Roach, 1950; Hurd and Kissinger, 1953), respectively; the twig tagging method (Aldous, 1944); and the photographic method (NAS-NRC, 1962).

Hand-Plucking

In situations where the determination of the grazing animal's diet is not possible or practical, hand-plucking of the pasture has been reported as an alternative method (Pieper et al., 1959; Edlefsen et al., 1960; Langlands, 1974). The technique consists of plucking the pasture by

one or more operators simulating the grazing animal (Corbett, 1978). A modification used in range research is to pluck material before and after the animals have access to the pasture (Cook, 1964). The method requires careful observation of animal preferences while grazing and (or) of the grazed plants and plant parts (Pieper et al., 1959). The major objection to the method is that the similarity of plucked samples to the material actually grazed is unknown (Corbett, 1978).

Hand-plucked samples were compared to esophageal fistula samples in estimating forage quality (Langlands, 1974). The main difficulties of its use were the overestimation of nutritive value at high levels of digestibility and N content and underestimation of the nutritive value when these were low. Statistically significant differences for all constituents except ether extract, protein and cellulose between hand-plucked and extrusa samples were reported by Edlefsen et al. (1960) and Campbell et al. (1968).

Although operators may be familiar with the diet of grazing animals, precision between hand-plucked samples and extrusa samples is low and may be increased by compositing samples obtained by a number of operators working independently (Langlands, 1974).

Analysis of Fecal Material

The basis for this method is the identification of fragments of plant cuticle in feces by microhistological examination (Sparks and Malechek, 1968). Fecal analysis provides advantages such as: 1) no restriction on animal movement or interference with the normal grazing habits of the animal (Crocker, 1959); 2) allows practically unlimited sampling (Ward, 1970) and 3) is practical when the same pasture is used by more than one animal species (Holechek et al., 1982). The method is particularly advantageous under range conditions where animals graze extensively on mixed plant communities and when studying endangered species (Anthony and Smith, 1974).

Some of the disadvantages of this method were discussed by Slater and Jones (1971), Smith and Shandruk (1979) and Sanders et al. (1980). These include: 1) low accuracy because the forage species may become unidentifiable in feces or are not in the same proportions as consumed, 2) it does not provide information on where the food was consumed, 3) considerable training is needed for accurate analysis, 4) analyses are tedious and time consuming and 5) special equipment is required for analysis.

Vavra et al. (1978) compared esophageal fistula samples and fecal material from cattle grazing shortgrass range in

Colorado. The authors reported that fecal analysis overestimated the occurence of grasses and underestimated the occurreace of forbs. When diets of pronghorn antelopes were estimated by rumen contents, intestinal feces, site feces and utilization estimates, fewer plants species were found in intestinal feces and site feces, compared to rumen contents. Fecal estimates overestimated the amount of grasses and shrubs and underestimated those of forbs (Smith and Shandruk, 1979). McInnis et al. (1983) reported that diets estimated by fecal and ruminal analysis were higher ($P<.05$) in grasses and lower ($P<.005$) in forbs than those estimated by esophageal fistulation and ocular estimates. The authors found similar results in a feeding trial where sheep were fed hand-composited mixtures of forages, and when grazing a common plant community.

A limitation to this method is the differential digestion of different plant species (Slater and Jones, 1971). Vavra and Holechek (1980) suggested the use of regression equations from in vitro digestion of hand-compounded mixtures of forages to overcome this problem. The accuracy of the method could be increased by the use of digestibility coefficients from various plant species, at different growth stages and for different animal species (McInnis et al., 1983).

An important feature of this technique is that a fecal sample represents the diet over more than a day, while esophageal or ruminal samples are collected a few times per day in less than an hour (Holechek et al., 1984).

Ruminal-Fistula Technique

Rumen fistula sampling has been used extensively to study botanical and chemical composition of animal diets (Norris, 1943; Lesperance et al., 1960a,b; Galt et al., 1969; Rice et al., 1971; McInnis et al., 1983). The technique requires surgical operation and special closure devices as described by McCann et al. (1973). The sampling procedure involves removal of all the rumen-reticulum contents and cleaning of the rumen walls. The animal is allowed to graze, the sampled material is removed and the initial rumen contents are replaced (Lesperance et al., 1960a). Botanical composition of the samples is determined by the microhistological technique (Sparks and Malechek, 1968) or by the microscope-point technique (Heady and Van Dyne, 1965). Higher ($P<.05$) proportions of grass species and lower ($P<.05$) proportions of shrubs and forbs were reported in rumen samples, compared to esophageal samples (Rice et al., 1971, McInnis et al., 1983). Rice et al. (1971) concluded that ruminal sampling leads to erroneous results because of the differential digestion of plant

species. Layering of rumen contents where grass portions are likely to float to the top of the rumen may bias results of ruminal-grab samples (Rice et al., 1971).

The advantages of the method are the direct sampling by the animal and that rumen samples contain all the forage consumed by the animal during the collection period (Lesperance et al., 1960b). The disadvantages of ruminal sampling are that it is limited to large animals, rumen evacuation may cause abnormal physiological conditions to the animal and it is very laborious (Rice, 1970).

Esophageal Fistula Sampling

The esophageal fistula technique was used as early as 1855 by Claude Bernard for physiological studies on horses (Van Dyne and Torell, 1964). However, its use in nutritional studies was begun by Torell in 1954. In early developmental stages, more animals were lost by post-operatory complications than by surgery techniques (Torell, 1954; Cook et al., 1958; McManus, 1962a,b). Improvements in surgical procedures, post-operative management and closure methods have minimized problems of establishing and maintaining fistulae (Corbett, 1978). Reviews on development and use of esophageal fistulae are those of Van Dyne and Torell (1964), Theurer (1970a) and Rice (1970). Descriptions of surgical

techniques have been published by Torell (1954), Cook et al. (1958), Hamilton et al. (1960), Chapman and Hamilton (1962), McManus (1962a,b) and Cook et al. (1963). A variety of closure devices have been reviewed by Torell (1964), McManus (1980) and by Holechek et al. (1982b). The pre- and post-surgical care of the animals was described by Cook et al. (1958) and by Hoene et al. (1965).

Some investigators pen the animals and keep them off feed overnight prior to collection periods (Bath et al., 1956; Kirby and Stuth, 1982; Holechek and Vavra, 1983). Reasons for this strategy are to reduce ruminal contamination and (or) induce animals to consume sufficient quantities of forages (Van Dyne and Heady, 1965). The duration of the fast affects the composition of the samples collected (Arnold et al., 1964; Sidahmed et al., 1977). Arnold et al. (1964) reported that sheep fasted for 17 h selected more of the most abundant botanical species. Fasting periods of 0, 12, 24 and 36 h in sheep grazing four different pastures showed that prolonged fasting (e.g., 36 h) may affect composition and in vitro digestibility of extrusa samples (Sidahmed et al., 1977). However, in this study the effect of fasting appeared to be a function of pasture type and was not the same for all chemical constituents. Fasting periods of up to 23 h had no effect on chemical composition of extrusa samples col-

lected from cattle (Cohen, 1979) and sheep (Langlands, 1967). Other workers allow the fistulated animals to graze and co-exist with the herd at all times (Arnold et al., 1964; Langlands, 1967; Cohen, 1969). Small herds of experimental animals were grazed together in other experiments (Cook et al., 1961; Van Dyne, 1964).

Arnold et al. (1964) and McManus (1981) suggested that animals should have prior grazing experience of the pastures under study. Langlands (1967) studied the differences in selectivity between sheep already grazing a pasture for 18 mo and sheep transferred 10 d before sampling. The N content of esophageal samples from sheep on pasture were 5% higher ($P<.01$) than those from sheep transferred. Other researchers place fistulated animals on pasture only a few days prior to sampling (Lesperance et al., 1960a; McInnis et al., 1983). Cohen (1979) concluded that lack of previous grazing experience of a pasture will not bias the estimation of the nutritive value of extrusa samples.

Collection periods vary between 15 min to 4 h, yielding samples of 20 to 300 g of dry matter for analysis (McManus, 1981). They depend on animal species, size of the fistula, rate of grazing, type and density of forage and experimental purposes (Van Dyne and Torell, 1964). Bath et al. (1956) suggested a maximum of 30 min for collections on irrigated

pastures while collection periods of 2 to 4 h were reported on winter ranges (Cook et al., 1958; Van Dyne, 1963). Sampling periods as short as 10 to 15 min have been used with sheep on small fenced plots (Lusk et al., 1961).

Time of sampling may be manipulated to reduce ruminal contamination (Holechek et al., 1982). Samples collected between 1000 and 1600 h had a greater chance of contamination, since steers usually ruminated at this time (Holechek et al., 1982). Other factors to consider when selecting the time of sampling are the daily and seasonal grazing habits of the animal (Arnold, 1962), and dietary changes due to pasture and weather changes or to animal satiation (McManus, 1981).

Diurnal variation in the nutritive value of the diet of esophageally-fistulated animals may bias the results of collections (Cohen, 1979). Higher N content in morning collections, compared to evening collections, were reported by several authors (McManus, 1961; Van Dyne and Lofgreen, 1964; Langlands, 1967; Hodgson, 1969; Obioha et al., 1970; Cohen, 1979; Kirby and Stuth, 1982; Holechek and Vavra, 1983). In vitro dry matter digestibility (IVDMD) of morning collections was higher ($P<.05$) than evening collections in a study by Langlands (1967). No difference in IVDMD was reported in similar work by other researchers (Cohen, 1979; Kirby and

Stuth, 1982; Holechek and Vavra, 1983). Obioha et al. (1970) and Holechek and Vavra (1983) reported higher lignin contents in morning, compared to evening collections. Differences in botanical compostion of morning collections, compared to afternoon collections have been observed (Obioha et al., 1970; Holechek and Vavra, 1983).

In spite of the differences reported, several studies showed that the between-animal or between-day variation were higher than diurnal variations, and have a greater effect on the results obtained (Weir and Torell, 1959; Lesperance et al., 1960b; Obioha et al., 1970). McManus (1981) suggested that collections should be made in the morning and afternoon, while Holechek and Vavra (1983) concluded that collections should be rotated between morning and afternoon, especially on pastures with heterogenous vegetation.

Several researchers have studied the number of fistulated animals required to obtain representative samples of pastures under grazing conditions (Van Dyne and Heady, 1965; Galt et al., 1969; Obioha et al., 1970; Harniss et al., 1975; Rosiere et al., 1975; Holechek and Vavra, 1983). Most of these studies showed that the number of diet samples needed for the determination of botanical composition was much greater than for the determination of nutritive value. Between six and eight animals are needed for proper sampling

in different pasture types: on semi-desert range (Rosiere et al., 1975); tall grass and mid-grass pastures (Obioha et al., 1970) and sandhill range (Wallace and Van Dyne, 1970). Van Dyne and Heady (1965) concluded that in order to estimate major individual species within 10% of the mean with 95% confidence, 24 animals are needed and up to nine animals are required for sampling at the forage class level (grass, shrubs and browse). Holechek and Vavra (1983) calculated that at least five animals and six collections (30 samples) are required for sampling at the forage class level, and four animals and four collections (16 samples) are needed to estimate nutritive quality of the diet at the same level of precision (10% of the mean and 95% confidence).

The estimation of lignin with similar precision requires a greater number of animals (Obioha et al., 1970; Wallace and Van Dyne , 1970; Rosiere et al ., 1975). Harniss et al. (1975) reported that 11 sheep were needed to estimate crude protein (CP), NDF and acid detergent fiber (ADF) on diets from sagebrush range. However, 30 sheep were required to estimate lignin with the same level of precision.

The principle problems with esophageal-fistula sampling are contamination with rumen contents and saliva, incomplete recoveries, low precision for estimating individual dietary species and high cost (Holechek et al., 1984).

Ruminal contamination of extrusa samples reduced the accuracy of chemical and botanical analyses (Holechek et al., 1982). Contaminations occur more frequently when collections are made at times that cattle normally ruminate. Data indicated that collections should be made according to the grazing habits of the animals in order to avoid contaminations. Van Dyne and Heady (1965) suggested that fasted animals graze more vigorously and do not contaminate the samples with regurgitated ingesta. However, prolonged fasting appeared to affect the composition of the samples collected (Sidahmed et al., 1977).

Collection periods longer than 30 min increase the chance of regurgitation of rumen contents into the collection bag (Bath et al., 1956). Holechek et al. (1982) reported that time of collection was more important than length of collection in preventing ruminal contamination.

Salivary contamination usually increases the ash content of extrusa samples (McManus, 1961; Arnold et al., 1966; Langlands, 1966; Mayland and Lesperance, 1977; Hart, 1983). Ash contamination is increased more with dry and fibrous feeds because such diets stimulate greater salivary flow (Kay, 1963).

Several researchers have studied the effect of mineral contamination of samples collected from esophageal fistulas

(Langlands, 1966; Hoehne et al., 1967; Little, 1972; Lesperance et al., 1974; Scales et al., 1974; Little, 1975; Mayland and Lesperance, 1977). These studies showed that salivary contamination increased the concentration of P, Na, Cl and Zn in extrusa samples, compared to the forage offered. Higher concentrations of Si and Co and small increases in K, Mn, Mo and Fe were reported by Mayland and Lesperance (1977). Calcium concentration appears to be the least affected by salivary contamination (Langlands, 1966; Little, 1975; Cohen, 1979). Small decreases in Ca contents of extrusa samples have been reported (Mayland and Lesperance, 1977; Hoehne et al., 1967). Salivary contamination has little effect on the N contents of extrusa samples (Lesperance et al., 1960a; Cundy and Rice, 1968; Barth et al., 1970; Little, 1972; Wallace et al., 1972; Mayland and Lesperance, 1977; Hart, 1983). Data from these studies suggest that total extrusa can be used to predict N content of grazed herbage. However, other researchers reported different N contents between extrusa samples and the forage consumed (Lesperance and Bohman,1964; Langlands, 1966; Hoehne et al., 1967; Campbell et al., 1968; Lesperance et al., 1974; Scales et al., 1974). In order to minimize N contamination of fistula samples, animals should be maintained on diets with N contents similar to that of the forage sampled (Mayland and Lesperance, 1977).

The process of mastication and salivation releases the contents of fractured plant cells (Grimes and Watkin, 1965). The higher fiber and lignin and lower soluble carbohydrates found in extrusa samples, compared to the original feed may be associated with loss of solubles (Hoehne et al., 1967; Lesperance et al., 1974). Other studies showed no changes in ADF and lignin between fistula samples and the feed consumed (Cundy and Rice, 1968). Wallace et al. (1972) reported no changes in ADF and lignin between untreated herbage and herbage soaked in saliva.

In vitro dry matter digestibility (IVDMD) of extrusa samples is affected by mastication and salivation (Langlands, 1966). Cundy and Rice (1968) reported lower IVDMD of fistula samples and forage samples soaked in saliva, compared to the forage offered to steers. The initial IVDMD of the forage, dropped from 60% to 49% and 47%, respectively.

Some of the common solutions to the problem of salivary contaminations are: 1) presentation of the data on an ash free basis (Grimes and Watkin, 1965; Wallace et al., 1972; Hart, 1983); 2) hand squeezing or draining of fistula samples (Hoehne et al., 1967; Lesperance et al., 1974; Acosta and Kothman, 1978); 3) rinsing of fistula samples with distilled water (Cundy and Rice, 1968; Hart et al., 1983; Hart, 1983); 4) correction factors because of the moisture added

to the forage by saliva (Van Dyne and Torell, 1964); 5) equations that account for the dry matter derived from saliva (Hart, 1983); 6) determination of the salivary contents of extrusas with tritiated water as a marker (Langlands and Bowles, 1973).

According to the research available, the most reliable solution to problems caused by salivary contamination is the presentation of the data in an ash free basis.

This problem has been studied by several researchers (Lesperance et al., 1960; Arnold et al., 1964; Grimes and Watkin, 1965; Hamilton and Hall, 1975). Percent recovery is usually, though not always, higher from larger size fistulae (Arnold et al., 1964; Blackstone et al., 1965). Studies showed that sample recovery ranged from 35 to 94% (Grimes and Watkin, 1965; Campbell et al., 1968). Hamilton and Hall (1975) reported recoveries greater than 35% of the diet ingested, but distal foam plugs increased recoveries up to 92%.

The chemical composition of fistula samples may be affected by sample preparation (Holechek et al., 1982). Squeezing or draining of liquids from extrusa samples in order to reduce salivary contamination, results in the loss of soluble nutrients affecting the chemical contents (Lesperance et al., 1974). Rinsing of fistula samples with distilled water reduces the N and IVDMD and usually increases

the fiber and lignin contents, compared to the original forage (Cundy and Rice, 1968; Hart, 1983).

Drying methods may affect the organic matter (OM) of extrusa samples (Lesperance et al., 1974). The principle components affected by oven drying at high temperatures are lignin and fiber (Van Dyne and Torell, 1964). Lesperance and Bohman (1964) reported higher lignin values for samples oven dried (65 C), compared to samples vacuum dried (25 C). A recent study by Holechek et al. (1982) showed that most researchers prefer to oven dry samples at a temperature of 50 C. Freeze drying of extrusas appears to give better estimates of the chemical constituents analyzed than air or oven drying (Smith et al., 1967; Acosta and Kothman, 1978). Acosta and Kothman (1978) compared extrusa samples that were freeze-dried, oven-dried (60 C) and air-dried (25 to 35 C). Freeze-dried samples were higher ($P<.05$) in total nonstructural carbohydrates (TNC) and hemicellulose, but lower in acid detergent lignin (ADL) than samples that were oven-dried or air-dried. However, other studies showed no differences between methods of drying for fiber and lignin contents of fistula samples (Cundy and Rice, 1968; Lascano et al., 1970).

Esophageal fistula samples may be frozen for storage when not dried immediately (Minson et al., 1976). Delaying the

freezing of samples for 4 h did not alter the chemical composition of samples (Acosta and Kothman, 1978).

Botanical Analysis of Extrusa Samples. The methods frequently used to determine the botanical composition of diet samples may be grouped into four categories: visual appraisal, manual separation, microscope point technique and microhistological examination (Holechek et al., 1984). Visual analyses without the aid of a microscope are suitable only for qualitative estimates of botanical composition (Theurer et al., 1976). Most browse plants in fistula samples may be identified by this method, but grasses and forbs are usually unidentifiable because of mastication effects (Cook et al., 1958). Because visual analysis is time consuming and has low precision, most researchers have preferred the microscope point and microhistological techniques (Holechek et al., 1982). These two techniques require different sample preparation and microscope magnifying power for proper identification of plant fragments.

Manual Separation. This method was studied by several researchers (Hoehne et al., 1965; Arnold et al., 1966; Hall and Hamilton, 1975; Marshall and Squires, 1979). The greatest source of error in the manual separation of plant fragments is the differential effect of mastication on plant parts (Minson et al., 1976). The microscope point technique

was more accurate than manual separation in studies comparing the two methods (Hall and Hamilton, 1975; Marshall and Squires, 1979).

The Microscope Point Technique. This technique was developed by Heady and Torell (1959) and described by Harker et al. (1964). It consists of identification of plant fragments with a binocular microscope of 16 X magnification and crosshairs in one eyepiece. The sample is placed on a movable tray which is passed under the microscope at pre-established stops. Between 100 and 400 points are read for each sample and the percent occurence is calculated for each plant species. Regression equations must be used to transform microscope hits into percent weight and are developed by analyzing diets of known composition (Heady and Van Dyne, 1965). The high correlations ($r \geq .85$) reported by several researchers indicate that this technique may be used to determine botanical composition (Holechek et al., 1982). Hamilton and Hall (1975) studied the error associated with the weight per unit area of plant components in leaves, petioles, flowers and seed pods of legumes. They suggested that the use of weight per area constants improved the estimation of the legume consumed. Theurer et al. (1976) suggested that microscope points should be related to volume rather than weight, because the regression equations are

less dependant on the species composition of the mixtures. Modifications of the method are those of Van Dyne and Heady (1965), Galt et al. (1969), Hamilton and Hall (1975) and Durham and Kothman (1977).

The Microhistological Technique. This method was first introduced by Baumgartner and Martin (1939) and refined by Dusi (1947). Sparks and Malechek (1968) verified and described this method where cuticle fragments are identified under a compound microscope with the aid of reference slides. In this study extrusa samples were oven-dried and ground through a 1 mm screen. Five slides were prepared for each sample, dried and analyzed under a compound binocular microscope. Each slide was read in 100 fields under a 125 X magnification. The frequency of occurence of different species was determined and converted to density, using the Fracker and Brischle table (Fracker and Brischle, 1979). Dry weight percentages were predicted from density. The ratio between estimated and actual dry weight was approximately 1:1. High correlations ($r \geq .95$) between observed and expected values for different forage mixtures have been reported (Sparks and Malechek, 1968; Vavra and Holechek, 1980; Holechek and Gross, 1982) Data presented by Holechek and Gross (1982) showed that the accuracy of the method was enhanced when observers were properly trained. They suggest-

ed that known mixtures should be used regularly by technicians in order to check their accuracy.

The number of slides and frequency of observations depends on the level of precision desired and the percent composition of the forage species in the diet (Holechek and Vavra, 1981). At least 20 observations per slide and five slides per sample were needed to give reasonable estimations.

The major problems associated with this technique were discussed by Holechek et al. (1984). The 1:1 ratio between density and percent weight that exists for some species may not be consistent for other species or growth stages (Havstad and Donart, 1978). Results from microhistological analysis may be influenced by the degree of identification of different species and plant parts (Holechek, 1982). Data from that study showed that sample preparation affects degree of identification of plant material and the accuracy of the method.

Another important factor affecting the results of this technique is the difference between observers in the identification of species (Westoby et al., 1976). Training of observers increased the accuracy of the technique (Holechek and Gross, 1982).

A recent study of the time and costs of microhistological analysis showed that a minimum of 4 h are required for the preparation, training and reading of 100 microscope fields per sample (Holechek et al., 1984).

Near Infrared Reflectance (NIR)

The NIR is a new technique with possible use in the determination of dietary botanical composition (Holechek et al., 1984). Botanical composition of grass:legume mixtures have been estimated to within 10% of the mean using this technique (Shenk et al., 1979). According to Holechek et al. (1984) more research is needed in the use and development of this technique.

Constituent Differential Method

This method was first developed by Cooper et al. (1957) to determine the species composition by weight of two-component forage mixtures. The method is based on different concentration of certain critical constituents in the two species. Moisture, CP, ADF, NDF and Ca concentrations have been compared in mixed hays and fresh grass-legume mixtures (Cooper et al., 1957; Johnson et al., 1982). The constituents are used in a formula:

$$(CG.G) + (CL.L) = CM$$

$$G+L=1.0$$

where:

G=proportion of grass in the mixture

L=proportion of legume in the mixture

CG=concentration of the component in the grass

CL=concentration of the component in the legume

CM=concentration of the component in the mixture

By rearrangement of this formula the proportions of grass and legume may be calculated :

$$G=(Cm-Cl)/(Cl-Cg)$$

If these calculations are repeated at different combinations of grass and legume, a linear model should result. A linear equation of the form $y=B_0+B_1x$ would express the replationship between the concentration of the constituents and the proportion of grass and legume. Finally, the proportion of grass and legume of any mixture may be calculated by extrapolation from such an equation.

The constituent differential method was used to determine the proportion of grass in samples from esophageally fistulated cattle grazing tropical pastures. The proportion of grass in the samples was determined by the microscope-point technique of Harker et al. (1964) and compared to those estimated from an equation with Ca as the main constituent. The best precision was obtained when known mixtures of extrusas were prepared, but these carried errors from the mi-

croscope-point estimations (Playne et al. 1978). The limitations found by the author were related to the differential Ca concentration in the plant parts of the tropical legume used. However, Ca appeared to be the ideal constituent to measure since it was not affected by mastication and salivation.

The constituent differential method yields best results when the pasture mixture contains only two species; the two species have a wide range in the concentrations of the constituent being analyzed; and when the concentration of the constituent does not vary greatly with plant parts, sampling site, soil fertility or stage of growth of the plant species (Playne et al., 1978).

Chapter III

RESEARCH

Objectives

The overall objectives of the present study were to assess feasibility of using chemical components to estimate botanical composition in grazed forages, specifically, to study the variation in concentration of Ca, neutral detergent fiber (NDF) and lignin in different grass:legume proportions and in extrusa samples of esophageally-fistulated steers and to determine the grass:legume proportions of extrusa samples from grazing animals.

Materials and Methods

Experiment 1. This experiment was conducted to study the variation in concentration of NDF, Ca and lignin in different grass:legume proportions. Forage samples were obtained from two pastures actively growing in late spring. The pasture species were mixtures of alfalfa (Medicago sativa L.) and tall fescue (Festuca arundinacea L.) in one field and red clover (Trifolium pratense) and orchardgrass (Dactylis glomerata L.) in the other. The alfalfa-fescue pasture was

harvested for hay a week before and the species were in vegetative stage. Red clover and orchardgrass were in flowering stage.

Eight .5 m^2 quadrats were located in each pasture. Stage of growth, proportions of grass, legume and weed, percent ground cover and dry matter yield were visually estimated by a panel of three persons for each quadrat. The forage in each quadrat was cut at a height of 2 cm. The material was collected in cloth bags, immediately frozen in dry ice, and stored in a freezer. The frozen material was hand separated in grass, legume, weed and dead material. Each fraction was thawed overnight, weighed and placed in a forced-draft oven at a maximum of 60 C for 24 h. All samples were ground to pass a 1 mm screen and stored in screw cap covered glass bottles. Samples of pure grass and pure legume were combined in the following proportions on a dry basis: 100:0; 80:20; 60:40; 40:60; 20:80 and 0:100.

Experiment 2. In order to obtain accurate predictions from the constituent differential method, calibration curves must be constructed. Curves developed from pure pasture samples may not be adequate to predict the botanical composition of extrusa samples. In order to obtain reliable results, mastication and salivation must not affect the concentration of the component nor the predictibility from

calibration curves. Based on the results of Experiment 1, this study was designed to examine if similar relationships exist between Ca and NDF concentrations in different grass:legume proportions and when they are fed to esophageally fistulated (EF) animals and to examine how the changes introduced by the animal affect linearity and predictibility of the components. Differences in these relationships could be caused by mastication and salivation. If these relationships are similar, it would be possible to use the extrapolation curves from the pastures to obtain the grass:legume proportions of the extrusa samples.

Two crossbred beef steers approximately 24 mo of age, and averaging 400 kg were used in an 8-d feeding trial. Esophageal fistulas had been successfully established 10 mo earlier according to the technique described by Ellis[1] et al. (1984). Animals were fed 3.6 kg/d of a maintenance diet, 4 wk prior to and during the experimental trial. The diet consisted of 55% orchardgrass hay (IFN 1-03-438), 40.2% ground corn (IFN 4-02-935), 3.5% soybean meal (IFN 5-04-604), .5% mineral supplement, .3% limestone, and vitamin A. Water and mineralized salts were available at all times. The animals were fasted for approximately 20 h before collections and were fed immediately after collections.

[1] Silicone cannulas and closure plugs were purchased from Texas A & M University

Red clover and tall fescue from pure stands growing in late spring were collected every afternoon for 8 consecutive days. Red clover was at 70% bloom and fescue was at head stage. The average heights of the legume and the grass were 56.5 and 83.7 cm, respectively.

On the afternoon prior to each feeding, 3 to 4 m^2 of red clover and tall fescue were harvested at approximately 1830 h. The harvested forage exceeded the amount needed for sampling and feeding. The forage was cut with a Gravely[2] scythe mower at an average height of 3.5 and 4.3 cm for the legume and grass, respectively. The material was then transported to the laboratory in a wood rack, weeds and extraneous material were removed by hand. The forage species were placed in separate racks, loosely covered with polyethylene and stored in a refrigerator overnight. On the next morning, four samples of 50 g each of the legume and grass were used for dry matter determination. The material was placed in a tared paper pan, weighed and placed in a microwave oven for 8 min. The procedure was repeated every 2 min until there was no change in weight, and the DM of the grass and legume was calculated.

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[2] Gravely Corp.

Six grass:legume combinations were prepared for each animal. The proportions of fescue and clover based on 100 g (dry basis) were: 100:0; 80:20; 60:40; 40:60; 20:80 and 0:100. The forage mixtures were placed in plastic buckets until feeding time. Subsamples of the pure species were stored in polyethylene bags, and frozen immediately for subsequent analysis.

Every morning prior to collection time, steers were individually penned and chained. Cannulas were removed to drain the esophagus and the fistulas were rinsed with deionized water. Feeding of the experimental forages started at approximately 1030 h, after adequate conditioning of the animals. The feeding schedule was obtained by randomly asigning treatments and days to each animal. The forage was fed in a trough, allowing 15 min intervals between treatments.

Extrusa samples were collected without loss of saliva in collection bags attached to the necks of the cattle with a harness. Collection bags were made from high density polyethylene. A different bag was used for each treatment. Contaminated or incomplete extrusa samples were discarded. Samples were stored in polyethylene bags and frozen immediatly in dry ice. Prior to chemical analysis, the frozen material was thawed at room temperature for approximately 12 h. Extrusa samples were placed in aluminum trays and dried in a

forced-draft oven at 50 C to a constant weight (approximately 4 d). Pure grass and legume samples were dried in a forced-draft oven at 50 C for 24 h. All samples were ground in a Wiley mill through a 1 mm screen and in screw cap covered glass bottles.

Experiment 3. This experiment was intended to study the use of the constituent differential method under grazing conditions and to predict the grass:legume proportions in extrusa samples. The same two esophageally-fistulated steers described in experiment 2 were used in a grazing experiment. One wk prior to the initiation of the experiment, a preliminary period of grazing on a similar pasture was conducted to familiarize the animals with the forage species and location. During this time the steers grazed the pasture for approximately 2 h every afternoon. The steers were then penned and fed 3.0 kg of the same maintenance diet used for Experiment 2. Water and mineralized salts were available at all times in the pens.

A 10-yr-old stand of tall fescue and red clover growing in late summer was used in this study. The grass was at vegetative stage and the clover at flowering stage. Two locations of approximately .5 ha each were selected within the pasture and each location was assigned to one steer. Each pasture was divided into 4 X 4 m plots and 10 plots were

randomly selected in each location and marked with stakes. Stage of growth, proportions of grass, legume and weeds, percent ground cover and dry matter yield were estimated visually for each plot by a panel of four persons.

For 2 consecutive d the animals were walked to the pasture with a halter at 1600 h to graze. The animals were fasted for approximately 20 h before collections and were fed the maintenance diet immediately after collections. Cannulas were removed to drain the esophagus and the fistulas were rinsed with deionized water. Animals were allowed to graze a given plot for 15 to 30 min. While one operator controlled the animal, samples were hand plucked by another person to simulate grazing.

An interval of approximately 5 min was allowed between each plot. Each animal grazed 5 different plots on each of the 2 d of the trial. Extrusa samples were collected without loss of saliva in collection bags attached to the necks of the cattle with a harness. Collection bags were made as described for Experiment 2. A different bag was used for each plot. Contaminated or incomplete extrusa samples were discarded. Pinch samples were taken daily after collections by clipping forage, every 5 steps following a zig-zag pattern in five strata of each location.

All samples were stored in plastic bags and frozen immediatly in dry ice. Prior to chemical analysis, the frozen material was thawed at room temperature for approximately 12 h. Extrusa samples were placed in aluminum trays and dried in a forced-draft oven at 50 C to a constant weight (approximately 4 d).

Chemical Analyses. For all experiments, forage samples were ground to pass through a 1 mm screen and stored in screw cap covered glass bottles. Dry matter (AOAC, 1980), NDF (Van Soest and Wine, 1967) Ca, and lignin (Van Soest and Wine, 1968) were determined in the samples. Samples of .5 g used for Ca analysis, were wet ashed with nitric and perchloric acids and diluted in a .5% (w/v) solution of lanthanum oxide. Calcium was determined by atomic absorption spectrophotometry (Perkin Elmer model 403) (Hern, 1979).

Statistical Analyses. Statistical analyses were performed using the regression analysis by the general linear model (GLM) procedure described by the Statistical Analysis System (SAS, 1982). In experiments 1 and 2, the constituents (Ca, NDF and lignin) were used as dependent variables, and the grass:legume proportion as the independent variable.

The constituent differential method allows the user to estimate the grass and legume proportions in mixtures by extrapolating values from appropriate regression curves

(Playne et al, 1978). Confidence intervals (CI) indicate the limits of the mean value of a given y at some specific value of x. Prediction intervals (PI) indicate the limits for an individual observation of y at a given value of x (Snedecor and Cochran, 1980). Therefore, in order to obtain reliable estimations, the extrapolated values should be accompanied by the upper and lower limits of the prediction intervals. Usually, a sample regression line is constructed by measuring y values at certain values of x. In this particular case, the line was used to predict x from y (called linear calibration).

In order to study the appropriatness of the simple linear regression (SLR) model, residuals were examined. Residual values are the difference between the observed values (yi) and those predicted by the model (Yi). If the model is appropriate, the residuals do not show a pattern when plotted against values of xi (Snedecor and Cochran, 1980).

Regression lines for the extrapolations for Experiments 2 and 3 were constructed by measuring the concentration of Ca and NDF at 0:100 and 100:0 grass:legume combinations.

The regression equations of the extrusa samples were compared to the equations for the pastures in order to examine the effects of mastication and salivation in experiment 2. If the pasture equations did not differ from the extrusa

equations, either curve could be used to extrapolate percent grass in the extrusa samples with the advantage that pasture curves are much easier to obtain. To compare two regression lines, the slopes (b_1) and intercepts (b_0) should be examined. For each day of the trial, regression equations were calculated for each animal and for the pastures, based on the Ca and NDF concentrations. The resulting intercepts and slopes were compared by the following contrasts: Animals 1 and 2 vs Pasture and Animal 1 vs Animal 2, for Ca and NDF concentrations.

In order to determine if the grab samples had a similar grass:legume proportion, compared to the extrusa samples, a paired t-test (alpha=.05 and df=12) was performed with the difference between % grass of the grab samples and of the extrusa samples. Similar values would indicate that grab sampling was effective in simulating the grazing animal.

The possibility of including NDF and Ca in a multiple regression equation was studied to determine if this would improve the estimations in experiment 2. The two variables (Ca and NDF concentrations) were examined by four multiple selection procedures: forward selection, backward elimination, stepwise regression and maximum R^2.

To study outliers the procedure followed was to omit these points from the regression analysis and compare the

results to the original analysis with all the data points. The extreme values that were examined were those that fell outside the 95% PI (appendix figures 7 to 12).

Results

Experiment 1. Visual estimations of the pastures were highly correlated with the actual values obtained by hand separation of the samples but not consistent through the components estimated. The r values also varied between pastures (table 1). The best correlation was obtained with DM yield in the fescue-alfalfa pasture (r=.98). However, the average values of the visual estimations and hand separations for DM yield were quite different but estimates of botanical composition were similar (table 2).

The chemical composition of the species are shown in table 3. Lignin and Ca concentrations in the legumes were 3.0 and 1.6 times higher than in grasses, respectively. The NDF values for grasses were 1.6 times greater than those of legumes.

Percent grass was regressed against Ca, NDF and lignin concentrations for each of the combinations. The resulting analysis of variance summaries are shown in appendix tables 11 and 12. The coefficients of determination (R^2) were

TABLE 1. CORRELATION COEFFICIENTS (r) BETWEEN VISUAL ESTIMATIONS AND HAND SEPARATIONS FOR FESCUE-ALFALFA AND ORCHARDGRASS-RED CLOVER PASTURES (EXPERIMENT 1)

Species	Component estimated			
	Grass	Legume	Weed	Dry matter yield
	---------- % ----------			kg/ha
Tall fescue-alfalfa	.63	.64		.98*
Orchardgrass-red clover	.91*	.88	.79	.52

*(P<.05)

TABLE 2. AVERAGE VALUES OF VISUAL ESTIMATIONS AND HAND SEPARATIONS FOR FESCUE-ALFALFA AND ORCHARDGRASS-RED CLOVER PASTURES (EXPERIMENT 1)

Item	Fescue-alfalfa		Orchardgrass-red clover	
	Visual estimation	Hand separation	Visual estimation	Hand separation
Grass, %	72.7	72.7	66.0	63.7
Legume, %	26.2	27.2	32.2	31.6
Weed, %	0.0	0.0	1.0	4.6
DM yield, kg/ha	560.0	1050.3	587.5	1876.2

TABLE 3. CHEMICAL COMPOSITION[a] OF THE SPECIES (EXPERIMENT 1)

Specie	Component[b]		
	Lignin	Neutral detergent fiber	Calcium
	---------- % ----------		
Tall fescue	4.41±.21	64.4±.57	.35±.01
Alfalfa	7.35±.42	37.9±1.41	.90±.02
Orchardgrass	4.53±.40	59.2±.70	.36±.003
Red clover	7.74±.69	38.9±.69	.97±.02

[a]Dry basis.

[b]Mean values±SE of the mean.

highest for Ca (.90 to .91), followed by NDF (.80 to .81) and lowest for lignin (.11 to .35) (table 4). The R^2 value represents the proportion of the total variation (SST) in the dependent variable (y) that is explained by the independent variable (x) of the model. The remainder corresponds to variation due to error (Ott, 1984). The R^2 values obtained showed that in the case of Ca and NDF the model accounted for more than 80% of the variation in percent grass. However, in the case of lignin the variance due to error was more than 70% of the total variability.

Another way of expressing the variability in the concentration of the components is the coefficient of variation (C.V.) (table 4). The C.V. for lignin were at least 30% higher than for Ca and NDF. A good attribute of C.V. as a measure of variation is that it allows a reliable comparison when different populations are used or when they have different units (Snedecor and Cochran, 1980).

The regression equations for each component are summarized in table 5. Since the components were regressed against percent grass, the slopes (b_1) for lignin and Ca were negative. As expected, higher proportions of grass resulted in lower concentrations of these two components. The opposite occured with NDF values. Plots of residuals vs values of xi (percent grass) are shown in appendix figures 1 to 6. No

TABLE 4. COEFFICIENTS OF DETERMINATION (R^2) AND OF VARIATION (C.V.) FOR THE GRASS AND LEGUME COMBINATIONS AND FOR THE THREE COMPONENTS (EXPERIMENT 1)

	Component					
	Lignin		Neutral detergent fiber		Calcium	
Species	R^2[a]	C.V.[b]	R^2	C.V.	R^2	C.V.
Fescue-alfalfa	.35	25.20	.81	9.15	.91	9.41
Orchardgrass-red clover	.11	50.47	.80	7.43	.90	11.54

[a]Coefficient of determination.

[b]Coefficient of variation, %.

TABLE 5. EQUATIONS RELATING PERCENT GRASS (x) TO CONCENTRATION OF COMPONENTS (y) AND THE 95% PREDICTION INTERVALS (EXPERIMENT 1)

Species	Component		
	Lignin	Neutral detergent fiber	Calcium
Tall fescue and alfalfa	y=7.17-.02x (±40.9)[a]	y=37.7+.26x (±14.5)	y=.91-.005x (±10.7)
Orchardgrass and red clover	y=7.69-.02x (±86.0)	y=39.1+.19x (±14.9)	y=.95-.006x (±9.0)

[a](P<.05).

clear patterns were observed in the data except in the Ca data. In this case a greater scatter of the points was found at 100% legume for both legumes tested. This variation in Ca concentration at only the 100% level is not sufficient evidence for transformations of the model variables. The simple linear model was the most appropriate in all cases, so there was no need for additional terms in the model.

The 95% CI and PI are shown in appendix figures 7 to 12. The 95% PI for Linear Calibrations shown in table 5 shows more evidence of the unreliability of lignin as an estimator. Obviously, if a certain value were extrapolated from the regression curves, a variation of 40 to 80% would be inadmissible (Snedecor and Cochran, 1980). The PI for Ca and NDF were much better than for lignin.

The regression analyses showed several extreme observations in the data sets. These points were examined in order to determine if they were true outliers and in what way they affected the results. The effects of omitting these data points were that the error term was reduced without significantly altering the slopes of the curves, meaning that these points were not important in the regression (appendix tables 13 and 14). It was concluded that these values were the product of experimental errors in sample handling and analysis.

Experiment 2. The average concentrations of Ca and NDF were .30 and 71.0%, and 1.13 and 42.7%, in tall fescue and red clover, respectively (table 6). The material recovered from the animals varied from 41 to 100% of the amount fed, with an average of 83.2%, which were similar or better than recoveries reported by other authors (Holechek et al., 1984). The concentrations of pure fescue and red clover recovered from the extrusas are shown in table 6. The differences in concentration were similar to those found in experiment 1 and in agreement with those reported by Holechek et al. (1984). The extrapolation equations were:

$$y=42.7 + .28x \quad \text{for NDF concentration}$$

and

$$y=1.13 - .008x \quad \text{for Ca concentration}$$

These models are presented graphically in appendix figures 13 and 14, and the equations are given in table 7. The equations were used to extrapolate values of percent grass from values of Ca and (or) NDF. The R^2 values were higher for the Ca data (.88 and .93) compared to the NDF data (.68 and .78) for animal 1 and animal 2, repectively (table 7). These differences in R^2 were expected and similar to the differences found in Experiment 1 between NDF and Ca.

The comparison of regression lines showed that the slopes and intercepts were not different between animals or between

TABLE 6.NEUTRAL DETERGENT FIBER (NDF) AND CALCIUM (Ca) CONTENTS[a] OF PURE FESCUE AND RED CLOVER AND RECOVERED FROM ESOPHAGEALLY FISTULATED ANIMALS (EXPERIMENT 2)

	Tall fescue		Red clover	
Item	NDF[b]	Calcium	NDF	Calcium
	%			
Pasture	71.0±.69[c]	.30±.01	42.7±1.12	1.13±.40
Extrusas				
Animal 1	74.2±.83	.27±.01	52.0±1.50	1.08±.07
Animal 2	74.0±.61	.27±.01	48.8±1.12	1.14±.03

[a]Dry basis.

[b]Neutral detergent fiber

[c]Mean±SE of the mean.

TABLE 7. EQUATIONS RELATING PERCENT GRASS (x) TO THE CONCENTRATION OF NDF AND Ca (y) IN PASTURES AND EXTRUSA SAMPLES AND THE THE CORRESPONDING COEFFICIENTS OF DETERMINATION (R^2) (EXPERIMENT 2)

Item	Neutral detergent fiber	Calcium
Pasture	y=42.7+.28x (1.0)[a]	y=1.13-.008x (1.0)
Extrusa		
Animal 1	y=51.4+.22x (.68)	y=1.05-.007x (.88)
Animal 2	y=50.5+.23x (.78)	y=1.11-.008x (.93)

[a]Coefficient of determination (R^2).

animals and pastures, when Ca was the component. When NDF was used, there were no differences between animals. However, the pasture line had a greater slope ($P<.05$) and a smaller intercept ($P<.01$) than the lines for the animals.

Prediction intervals are shown in table 8. The values for the pasture lines were greater than for the animals because of the fewer observations in the regression. The PI for the extrusa curves were similar to those found in Experiment 1. The multiple variable selection procedures are given in appendix table 16. The variable Ca was the best when only one variable was in the model ($R^2=.907$ and SSE=9,088), compared to NDF ($R^2=.759$ and SSE 23,609). When NDF was added to the model, the R^2 was increased by less than 1% (.907 and .914 in Ca alone and Ca+NDF, respectively) and the t-value for the NDF regression coefficient was not significant. These results indicate that the model was not improved by including both variables.

Experiment 3. According to the visual estimations the botanical composition of the pastures were: 77% grass, 17% legume and 5% weeds. The species covered 95% of the ground with an average height of 20.7 cm and a DM yield of approximately 700 k/ha (appendix table 15). The amount of forage collected via esophageal fistula from the animals ranged from 249 to 581 g of fresh material, with an average DM of

TABLE 8. PREDICTION INTERVALS[a] FOR VALUES EXTRAPOLATED FROM NEUTRAL DETERGENT FIBER AND CALCIUM CURVES (EXPERIMENT 2)

Item	Neutral detergent fiber	Calcium
Pasture	±15.8	±18.6
Extrusa		
Animal 1	±19.6	±11.7
Animal 2	±14.9	± 8.5

[a](P<.05).

11.8%. Seven of the 20 extrusa samples were discarded because the animals grazed weeds. This was determined visually while the animals grazed and by visual observation of the grab samples.

Tall fescue obtained by hand sampling had 59.2% NDF and .38% Ca. The composition of red clover was 47.7% NDF and 1.09% Ca. These values were used to construct the following extrapolation curves: NDF=47.7 +.11x and Ca=1.09 -.007x (x=%grass) to estimate the percent grass in the extrusa samples and grab samples. The extrapolated values are shown in table 9. Grass:legume proportions of grab samples differed ($P<.01$) from extrusa samples for NDF and Ca extrapolations.

Since grab samples were not adequate estimators of the animal's grazing habits, simple correlation was performed between the four variables to study their linear relationship. Percent grass from grab samples and extrusa samples for Ca and NDF extrapolations were compared. The results showed that the correlation coefficients (r) were low in all cases, except for grab and extrusa samples for Ca (r=.74) (table 10). In this case, the significant r value suggests that both variables were closely related.

TABLE 9. PERCENT GRASS IN EXTRUSA SAMPLES AND GRAB SAMPLES OBTAINED BY EXTRAPOLATION FROM NEUTRAL DETERGENT FIBER (NDF) AND CALCIUM (Ca) CURVES (EXPERIMENT 3)

Plot [a]	Grab samples		Extrusa samples	
	NDF[b]	Calcium	NDF	Calcium
1	18.0	65.9	70.9	62.3
5	40.0	53.7	101.2	36.5
6	12.6	43.2	42.9	23.4
8	36.0	75.8	92.4	65.2
9	33.8	85.8	147.4	76.3
10	18.6	58.3	117.6	30.0
11	41.6	55.4	84.6	64.5
14	42.7	66.2	113.2	32.8
15	20.8	57.6	89.6	37.6
16	63.5	84.7	144.2	60.0
17	13.7	60.7	49.2	47.6
18	71.8	57.5	100.2	42.3
20	17.9	93.2	117.3	64.5

[a]Plots 1 to 10 correspond to Animal 1. Plots 11 to 20 correspond to Animal 2.

[b]Neutral detergent fiber.

TABLE 10. CORRELATION COEFFICIENTS (r) BETWEEN ESTIMATIONS OF PERCENT GRASS IN GRAB SAMPLES AND EXTRUSA SAMPLES FROM NEUTRAL DETERGENT FIBER AND CALCIUM CURVES (EXPERIMENT 3)

Item	Grab samples		Extrusa samples
	Neutral detergent fiber	Calcium	Calcium
Extrusas			
NDF data	.49	.68*	.37
Ca data	.14	.74**	
Grab samples			
Ca data	.14		

*Significant at the (P<.05) level.

**Significant at the (P<.01) level.

Discussion

The techniques available for the determination of the botanical composition of extrusa samples have serious limitations. Among them are the low accuracy and precision due greatly to the subjectivity of the analyses, the time involved in each analysis, and the special training and equipment required.

The results of the present study showed that the concentrations of Ca and NDF were comparable to those reported for the species and mixtures reported previously (NRC, 1982). However, small differences in concentration were found due in part to different growth stages of a given species when used in different experiments. Since wider differences between grasses and legumes permit more precise predictions, the data suggest that Ca would be the most appropriate constituent, which agrees with studies by Playne et al. (1978).

The present experiment showed that the Constituent Differential Method is a promising technique and agreed with the results of Johnson et al. (1982) who estimated the grass:legume proportions in different mixtures of alfalfa, red clover, and ladino clover mixed with tall fescue, with NDF as the critical constituent. They reported that concen-

tration of NDF was approximately 20 units greater in tall fescue, compared to the legumes, and R^2 values between known and predicted values ranged from .92 to .98, similar to the values obtained in the present study. In the present experiments Ca was 3.0 times as high in legumes than in grasses. Based on prediction equations and correlations, Ca is a good predictor of proportions of grasses and legumes. These results agree with those of Playne et al. (1978).

The proportion of grass in esophageal fistula samples from cattle grazing a pasture of tropical grasses and stylo (Stylosanthes sp.) was estimated by Ca analysis and compared to estimations by the microscope-point technique (Playne et al., 1978). In the present studies lignin concentration varied greatly between replications of the same samples and therefore were not a reliable source of data. The most crucial problems for lignin determinations were analytical variations. The differences in concentration between grasses and legumes appeared to be sufficient to expect good predictions of the grass:legume proportions based on any of the three components.

The calibration of the method (experiment 1) showed that the concentration of the three components were linearly related with the variation in the grass:legume proportions. The linear relationships between grass:legume proportions

and the concentrations of the components were very strong as shown by the high R^2 values, except in the case of lignin. Similar relationships and R^2 values were obtained when grass:legume mixtures were fed to esophageally-fistulated steers in experiment 2, which showed that linearity was not changed by mastication and salivation. The R^2 values were similar or superior to those reported by Playne et al. (1978), in all cases except lignin.

Concentration of NDF in extrusa samples was greater than in the original material fed. This was shown by the difference in slope and intercept of the corresponding equations. The greater concentration of fiber in the extrusa samples was also observed by Hoehne et al. (1967) and Lesperance et al. (1974). The differences in NDF concentration were probably due to mastication and salivation resulting from leaching of solubles and enzyme activity (Grimes and Watkin, 1965) or the effects of the drying method (Van Dyne and Torell, 1964), which is not likely since the samples were dried at 50 C. Since the extrusa samples had higher NDF concentration than the pastures, extrapolations from the corresponding equations would differ. Values extrapolated from the fresh forage curves overestimated the amount of grass in the diet, compared to those calculated from curves obtained from the animals. This did not occur with Ca where

the percent grass was similar when estimated from either curve, with the advantage that pasture curves are easier and faster to obtain. The process of mastication and salivation did not affect the Ca concentration of extrusa samples, which is in agreement with other workers (Langlands, 1966; Little, 1975; Cohen, 1979; Playne, 1978).

The equations for tall fescue-red clover were not identical in experiments 2 and 3, probably because of the different growth stages of the species. Obviously, the best equations for a given pasture or grazing situation would result from calibrations of known combinations of the species when recovered from esophageally-fistulated animals. However, estimations from pasture equations would be similar and equally reliable. The possibility of using a pasture equation in other sites and at different times than when it was originated, depends on several factors: the same species must be used; the concentration of the constituent should be as similar as possible in the same species; the differential concentration of the constituent between the grass and legume must be maintained. For this purpose, the concentration of the constituent should be checked in the pasture species from the location before fistula sampling. In fact, concentrations would be used to create a new pasture equation each time. If new equations are similar to the 'origi-

nal' equation, they may be used on extrusa samples. Standard equations can be calculated for a combination of species after calibration and regression analysis are performed.

Results from this study showed that grab samples should not be used to estimate the diet of the grazing animal, which is in agreement with previous studies (Edlefsen et al., 1960; Campbell et al., 1968). The estimation of the grass:legume proportions in extrusa samples by the Constituent Differential Method would yield important information concerning the diet of grazing animals where much of the forage is not grazed. In order to obtain best results, the pasture should contain two species; the concentration of the constituent should differ greatly in the two species and ideally the concentration of the chemical should not vary greatly in different plant parts, at different sampling sites, or with soil fertility or growth stage of the plants (Playne et al., 1978). Also, samples should not be affected greatly by mastication and salivation.

Conclusions

Considering the wide differences in concentration of the constituents between grasses and legumes, it may be assumed

that the three constituents analyzed would be appropriate for reliable estimations. The widest concentration ratio between grasses and legumes was obtained with Ca (1:3), compared to 1:1.6 for lignin, and 1:1.6 for NDF.

The concentrations of Ca, lignin and NDF were linearly related to the proportion of grass and legume. However, the large analytical error associated with lignin determinations precluded its use to obtain reliable estimates. The concentration of Ca and NDF were linearly related to the proportions of grass and legume in forage mixtures recovered from esophageally fistulated steers. There was no effect of mastication and salivation on the concentration. However, extrusa samples had greater NDF concentration than the original pastures. Therefore extrapolations from pasture curves were better for Ca than for NDF.

The method under study appeared to be practical and simple to use. Routine chemical analysis and simple statistical procedures were needed to estimate the grass:legume proportions in a pasture mixture and in extrusa samples.

Of the constituents examined in this study, Ca was the most reliable constituent because of its good predictibility and not being affected by the animal. Fiber was a good predictor but was affected by mastication and salivation of the animal.

The Constituent Differential Method, as proposed here, requires more study. Some of the factors that were not studied were the effect of weeds and other forage species in the diet, the changes in concentration of the constituents in different plant parts, the effects of sampling site, soil fertility, and stage of growth.

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APPENDIX

TABLE 11. ANOVA[a] SUMMARIES OF THE LINEAR REGRESSION OF LIGNIN, NDF, AND Ca AGAINST PERCENT GRASS FOR ORCHARDGRASS-RED CLOVER COMBINATIONS (EXPERIMENT 1)

	Parameters				
Item	df[b]	SS[c]	MS[d]	R^2[e]	C.V.[f]
Lignin					
Model	1	133.11	133.11	.10	50.47
Error	110	1081.16	9.82		
Total	111	1214.27			
Neutral detergent fiber					
Model	1	5972.14	5972.14	.80	7.43
Error	110	1459.31	13.26		
Total	111	7431.45			
Calcium					
Model	1	5.57	5.57	.89	11.54
Error	110	.62	.003		
Total	111	6.19			

[a]Analysis of variance.

[b]Degrees of freedom.

[c]Sum of squares.

[d]Mean squares.

[e]Coefficient of determination.

[f]Coefficient of variation.

TABLE 12. ANOVA[a] SUMMARIES OF THE LINEAR REGRESSION OF LIGNIN, NDF, AND Ca AGAINST PERCENT GRASS FOR TALL FESCUE-ALFALFA COMBINATIONS (EXPERIMENT 1)

	Parameters				
Item	df[b]	SS[c]	MS[d]	R^2[e]	C.V.[f]
Lignin					
Model	1	118.73	118.73	.34	25.20
Error	107	221.20	2.06		
Total	108	339.93			
Neutral detergent fiber					
Model	1	10191.55	10191.55	.81	9.15
Error	110	2370.09	21.54		
Total	111	12561.64			
Calcium					
Model	1	3.45	3.45	.91	9.41
Error	94	.33	.003		
Total	95	3.78			

[a]Analysis of variance.

[b]Degrees of freedom.

[c]Sum of squares.

[d]Mean squares.

[e]Coefficient of determination.

[f]Coefficient of variation.

TABLE 13. ANOVA[a] SUMMARIES OF THE LINEAR REGRESSION OF LIGNIN, NDF, AND Ca AGAINST PERCENT GRASS FOR ORCHARDGRASS-RED CLOVER COMBINATIONS WITHOUT OUTLIERS (EXPERIMENT 1)

	Parameters				
Item	df[b]	SS[c]	MS[d]	R^2[e]	C.V.[f]
Lignin					
Model	1	59.91	59.91	.11	38.50
Error	102	462.65	4.53		
Total	103	522.57			
NDF					
Model	1	6188.20	6188.20	.85	6.52
Error	107	1087.92	10.16		
Total	108	7276.12			
Ca					
Model	1	4.46	4.46	.94	7.75
Error	101	.23	.002		
Total	102	4.69			

[a]Analysis of variance.
[b]Degrees of freedom.
[c]Sum of squares.
[d]Mean squares.
[e]Coefficient of determination.
[f]Coefficient of variation.

TABLE 14. ANOVA[a] SUMMARIES OF THE LINEAR REGRESSION OF LIGNIN, NDF, AND Ca AGAINST PERCENT GRASS FOR TALL FESCUE-ALFALFA COMBINATIONS WITHOUT OUTLIERS (EXPERIMENT 1)

	Parameters				
Item	df[b]	SS[c]	MS[d]	R^2[e]	C.V.[f]
Lignin					
Model	1	112.37	112.37	.40	22.31
Error	103	164.98	1.60		
Total	104	277.35			
Neutral detergent fiber					
Model	1	9883.65	9883.65	.84	8.24
Error	106	1880.40	17.73		
Total	107	11764.05			
Calcium					
Model	1	3.27	3.27	.94	7.26
Error	89	.19	.002		
Total	90	3.46			

[a]Analysis of variance.

[b]Degrees of freedom.

[c]Sum of squares.

[d]Mean squares.

[e]Coefficient of determination.

[f]Coefficient of variation.

TABLE 15. MEAN VALUES OF VISUAL ESTIMATIONS OF 20 PLOTS OF FESCUE AND RED CLOVER (EXPERIMENT 3)

Plot	Parameters[ab]				
	Grass	Legume	Weed	Ground cover	DM Yield
	%				kg/ha
1	80	15	5	95	953
2	72	24	4	95	707
3	71	24	5	96	770
4	67	21	11	96	1,261
5	70	24	6	96	560
6	69	19	12	96	826
7	89	7	4	94	700
8	82	12	5	92	476
9	94	1	5	96	813
10	84	11	5	96	574
11	76	16	7	95	630
12	80	15	5	97	574
13	87	9	4	96	504
14	69	27	4	96	602
15	57	34	9	96	714
16	81	7	11	96	560
17	65	34	1	94	579
18	66	31	2	96	728
19	90	10	0	100	841
20	95	3	2	96	678

[a]Average of a panel of four persons.

[b]Dry basis.

TABLE 16. ANOVA[a] OF THE MULTIPLE VARIABLE SELECTION PROCEDURES FOR NDF AND Ca VARIABLES (EXPERIMENT 2)

Item	df[b]	SS[c]	MS[d]	R^2[e]
Forward Selection				
Stepwise Regression				
Maximum R^2				
Ca entered				
Regression	1	88911.7	88911.7	.907
Error	82	9088.3	110.8	
Total	83	98000.0		
NDF entered				
Regression	2	89633.9	44816.9	.914
Error	81	8366.0	103.2	
Total	83	98000.0		
Backward Elimination				
Ca and NDF entered				
Regression	2	89633.9	44816.9	.914
Error	81	8366.0	103.2	
Total	83	98000.0		

[a]Analysis of variance.

[b]Degrees of freedom.

[c]Sum of squares.

[d]Mean squares.

[e]Coefficient of determination.

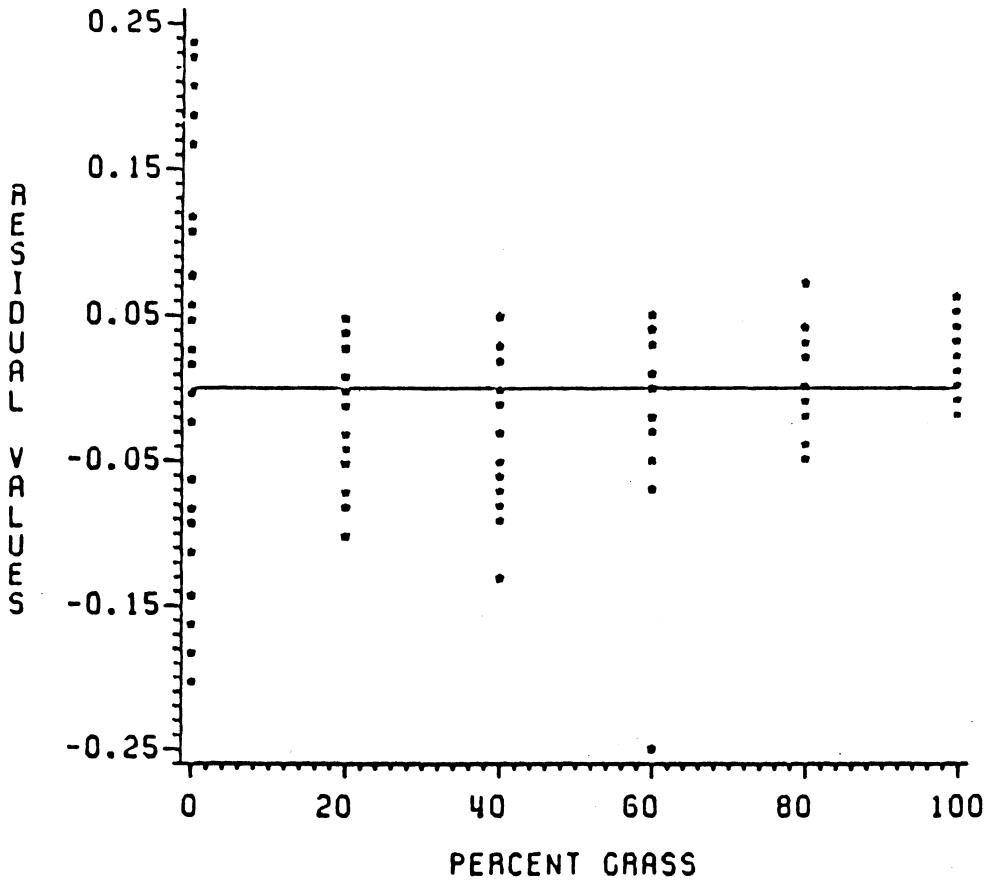

Figure 1: PLOT OF RESIDUALS OF Ca CONCENTRATION VS VALUES OF PERCENT GRASS FOR ORCHARDGRASS-RED CLOVER MIXTURES (EXP. 1)

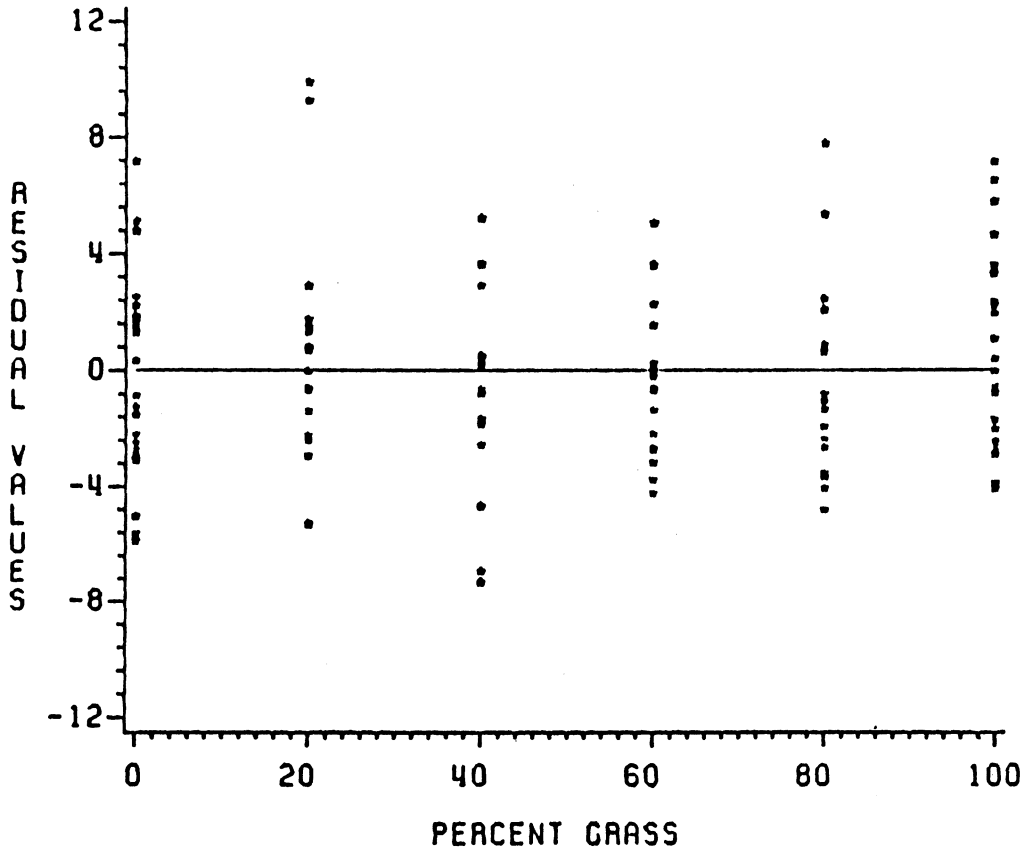

Figure 2: PLOT OF RESIDUALS OF NDF CONCENTRATION VS VALUES OF PERCENT GRASS FOR ORCHARDGRASS-RED CLOVER MIXTURES (EXP. 1)

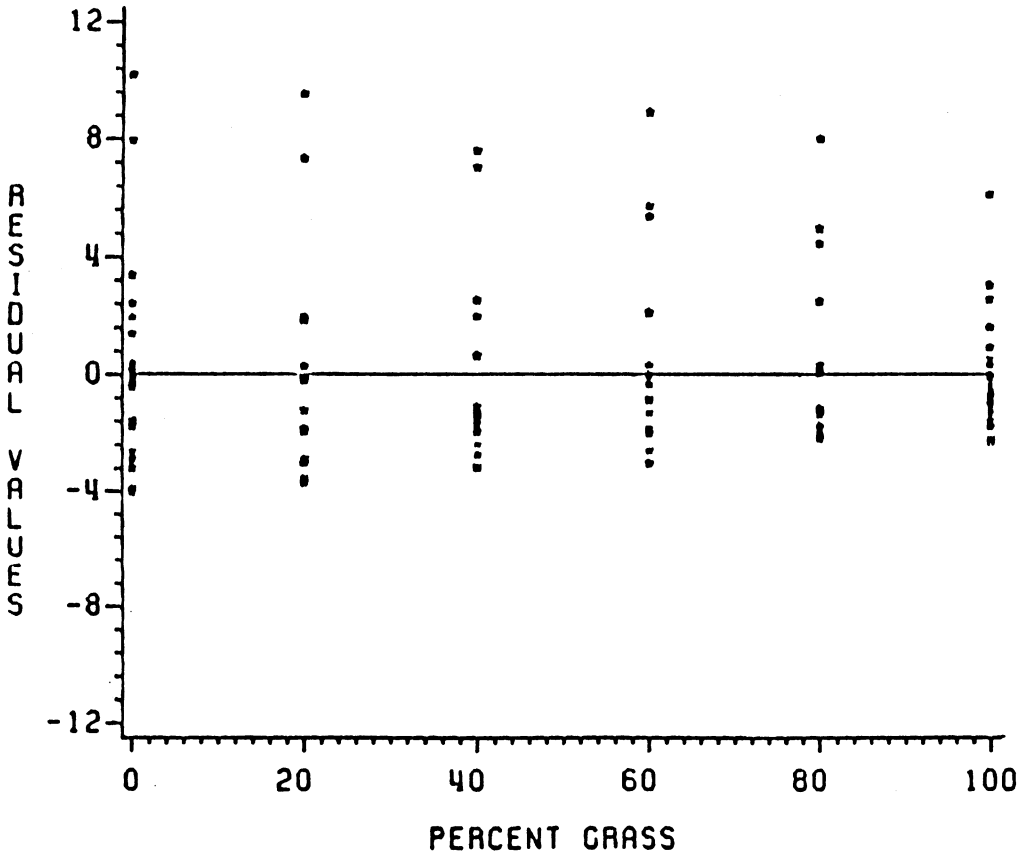

Figure 3: PLOT OF RESIDUALS OF LIGNIN CONCENTRATION VS VALUES OF PERCENT GRASS FOR ORCHARDGRASS-RED CLOVER MIXTURES (EXP. 1)

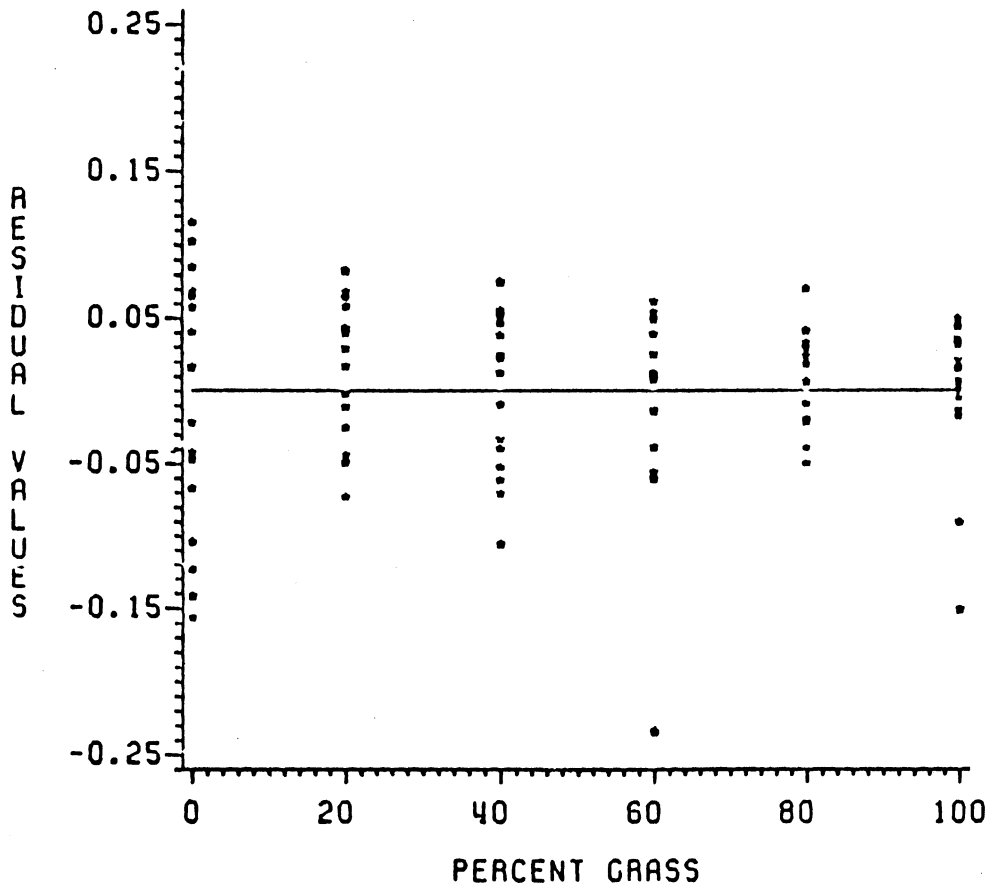

Figure 4: PLOT OF RESIDUALS OF Ca CONCENTRATION VS VALUES OF PERCENT GRASS FOR FESCUE-ALFALFA MIXTURES (EXP. 1)

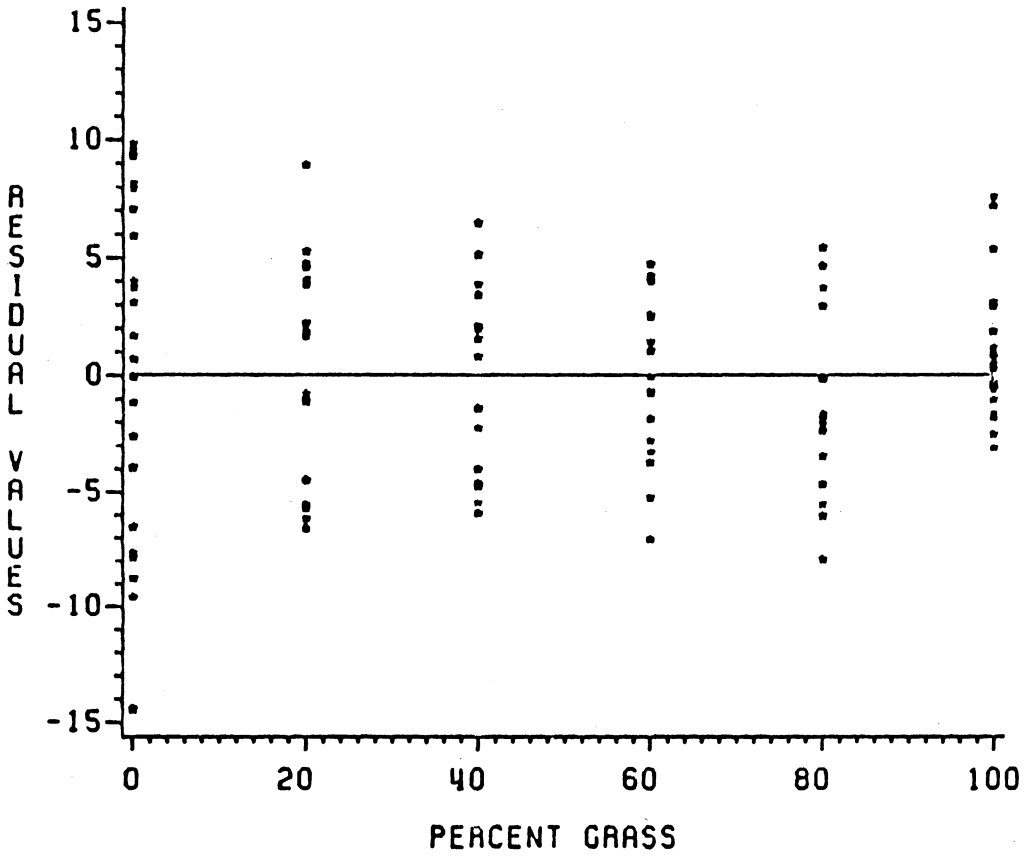

Figure 5: PLOT OF RESIDUALS OF NDF CONCENTRATION VS VALUES OF PERCENT GRASS FOR FESCUE-ALFALFA MIXTURES (EXP. 1)

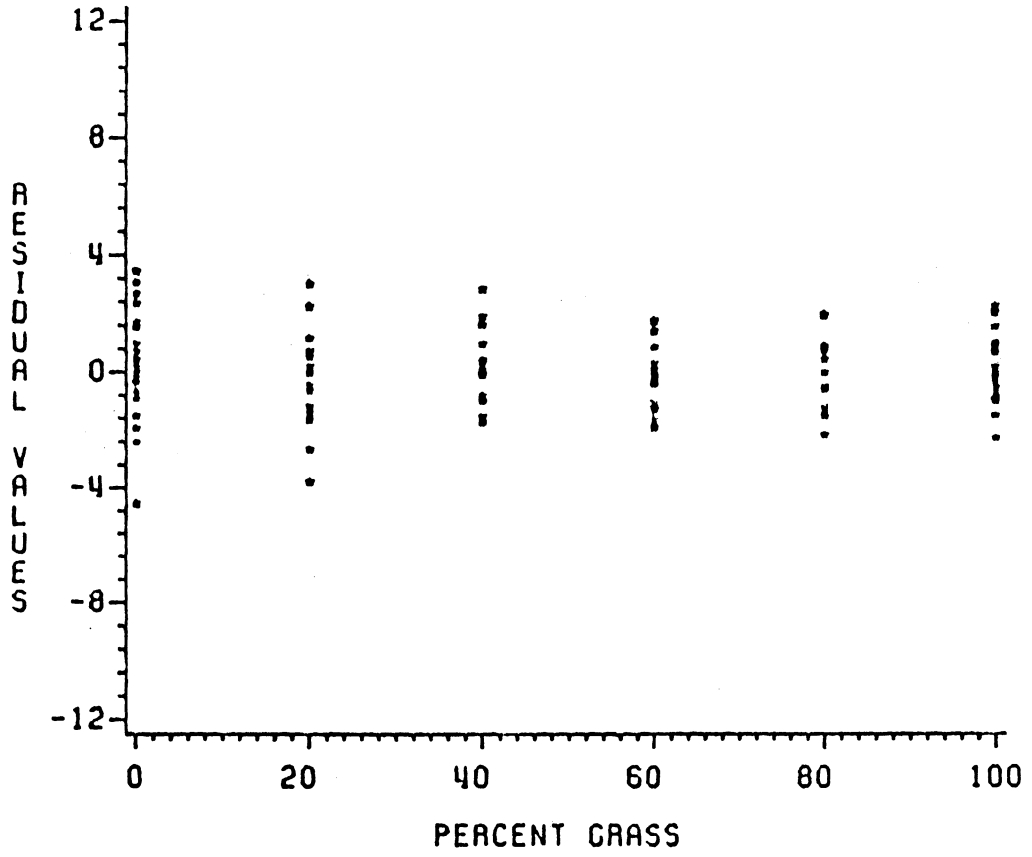

Figure 6: PLOT OF RESIDUALS OF LIGNIN CONCENTRATION VS VALUES OF PERCENT GRASS FOR FESCUE-ALFALFA MIXTURES (EXP. 1)

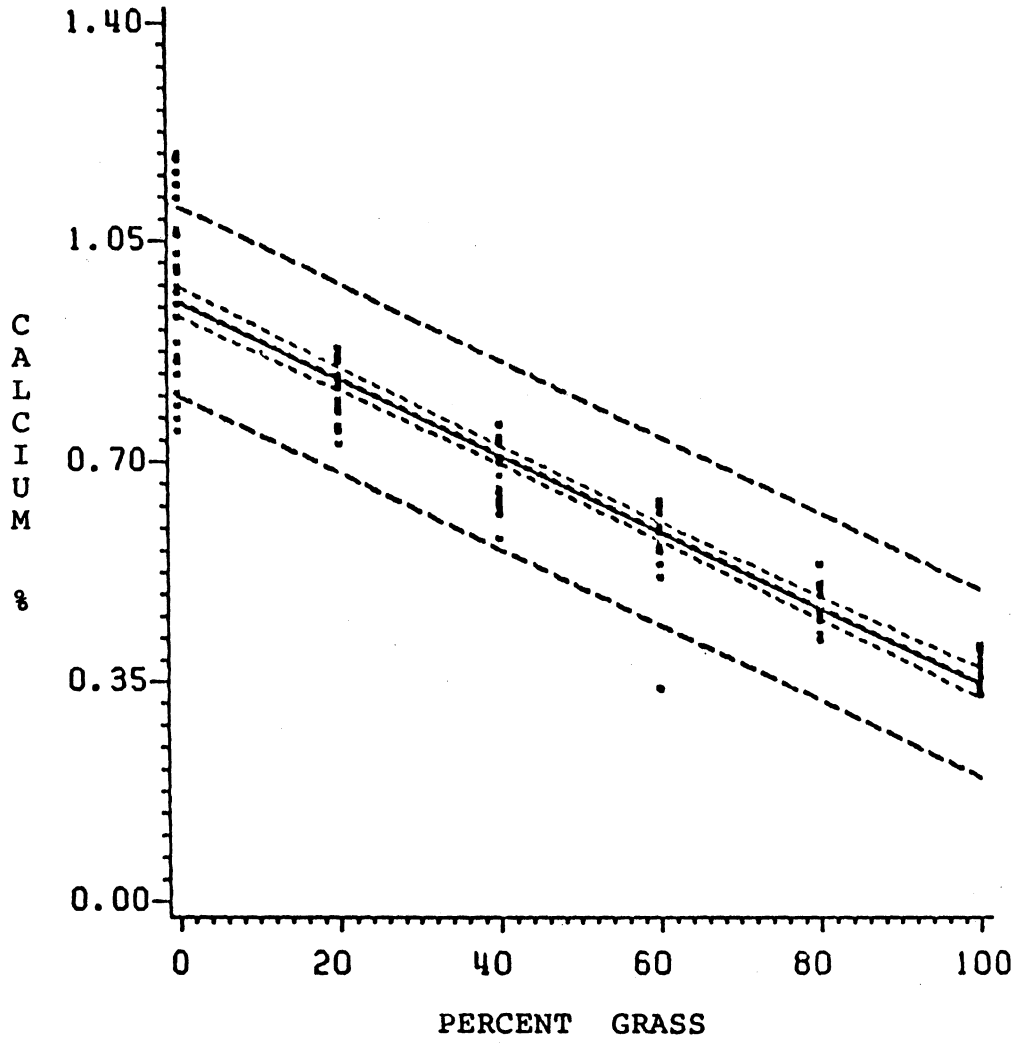

Figure 7: REGRESSION LINE (__), 95% CONFIDENCE LIMITS (---) AND PREDICTION LIMITS (—–) FOR Ca IN ORCHARDGRASS-RED CLOVER (EXP. 1)

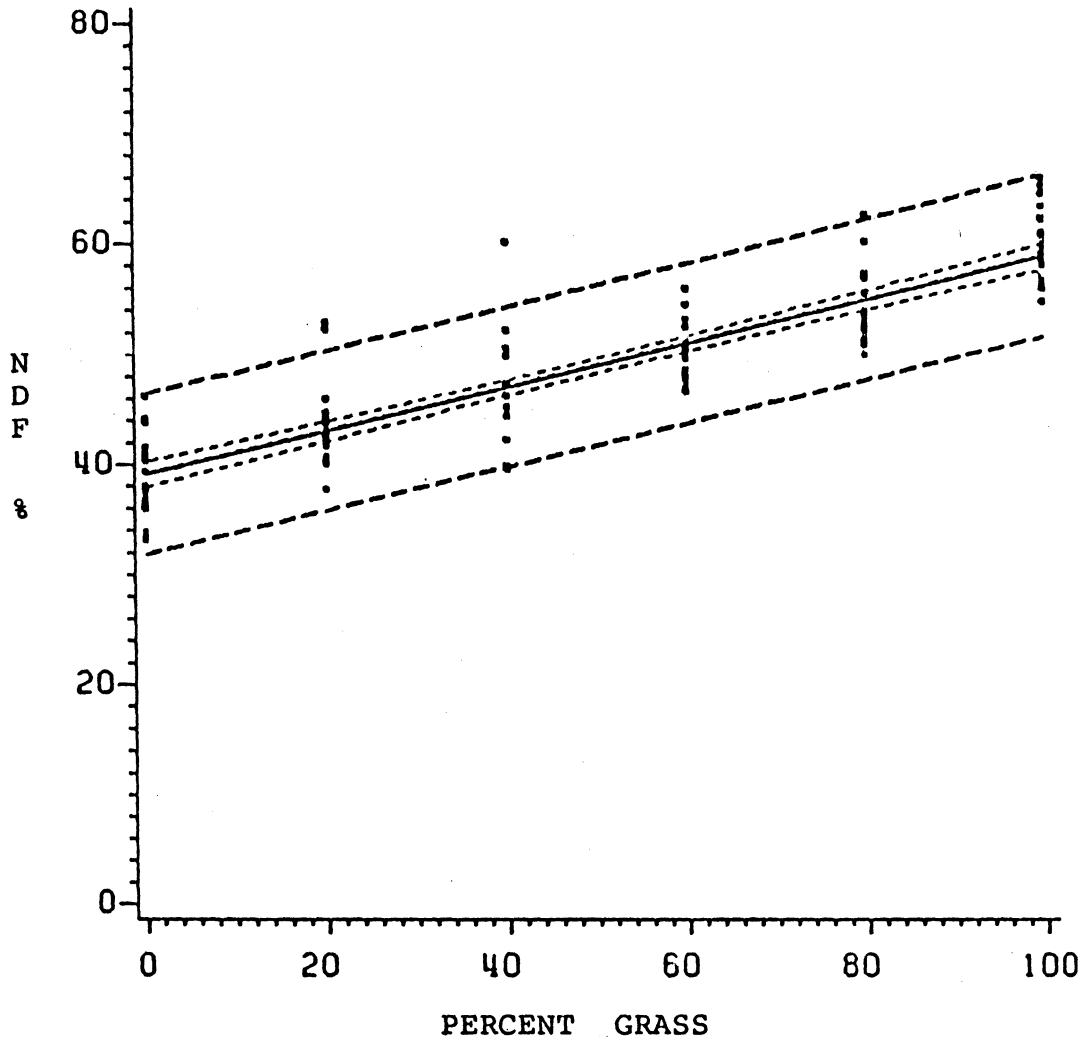

Figure 8: REGRESSION LINE(__), 95% CONFIDENCE LIMITS(--) AND PREDICTION LIMITS(—–) FOR NDF IN ORCHARDGRASS-RED CLOVER (EXP. 1)

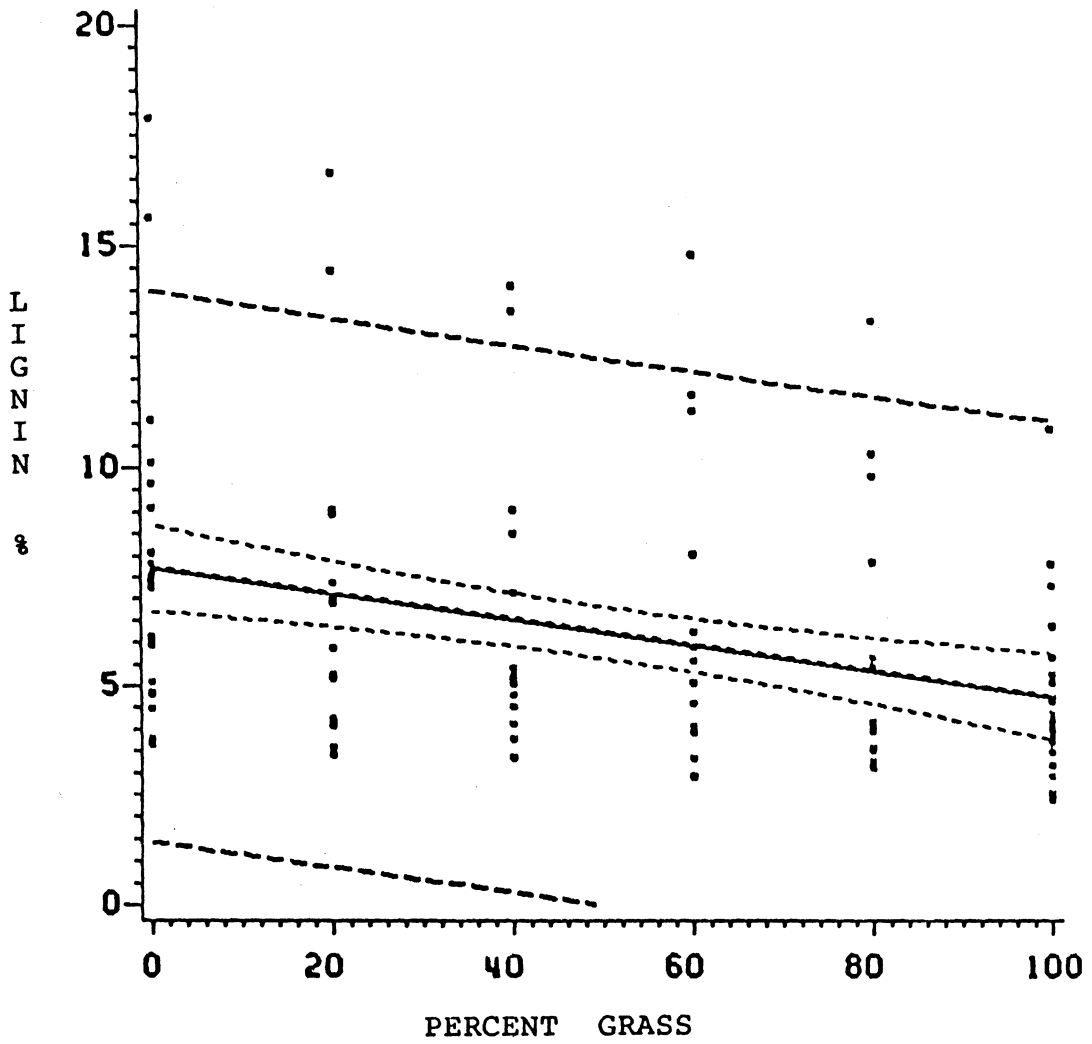

Figure 9: REGRESSION LINE(__), 95% CONFIDENCE LIMITS(--) AND PREDICTION LIMITS(—-) FOR LIGNIN IN ORCHARDGRASS-RED CLOVER (EXP. 1)

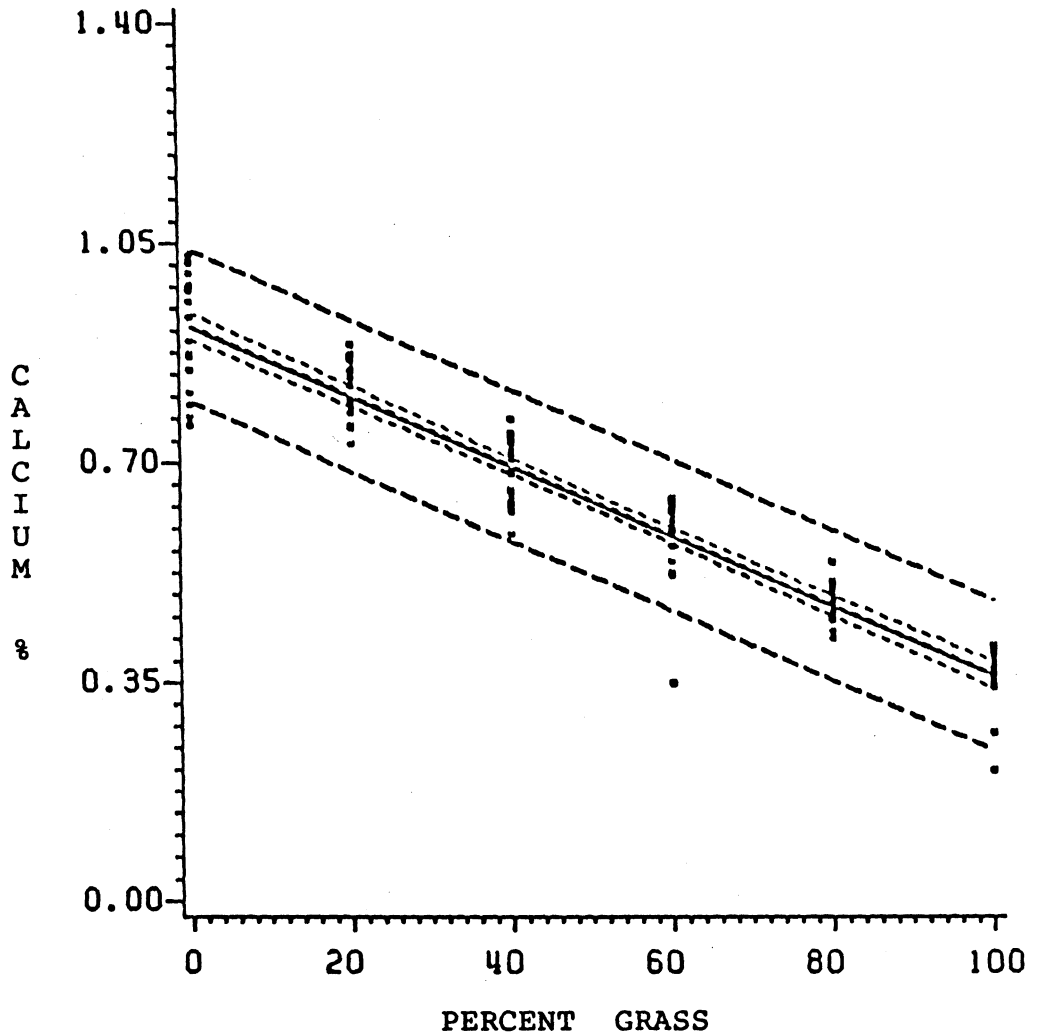

Figure 10: REGRESSION LINE(__), 95% CONFIDENCE LIMITS(--) AND PREDICTION LIMITS(—–) FOR Ca IN FESCUE-ALFALFA MIXTURES (EXP.1)

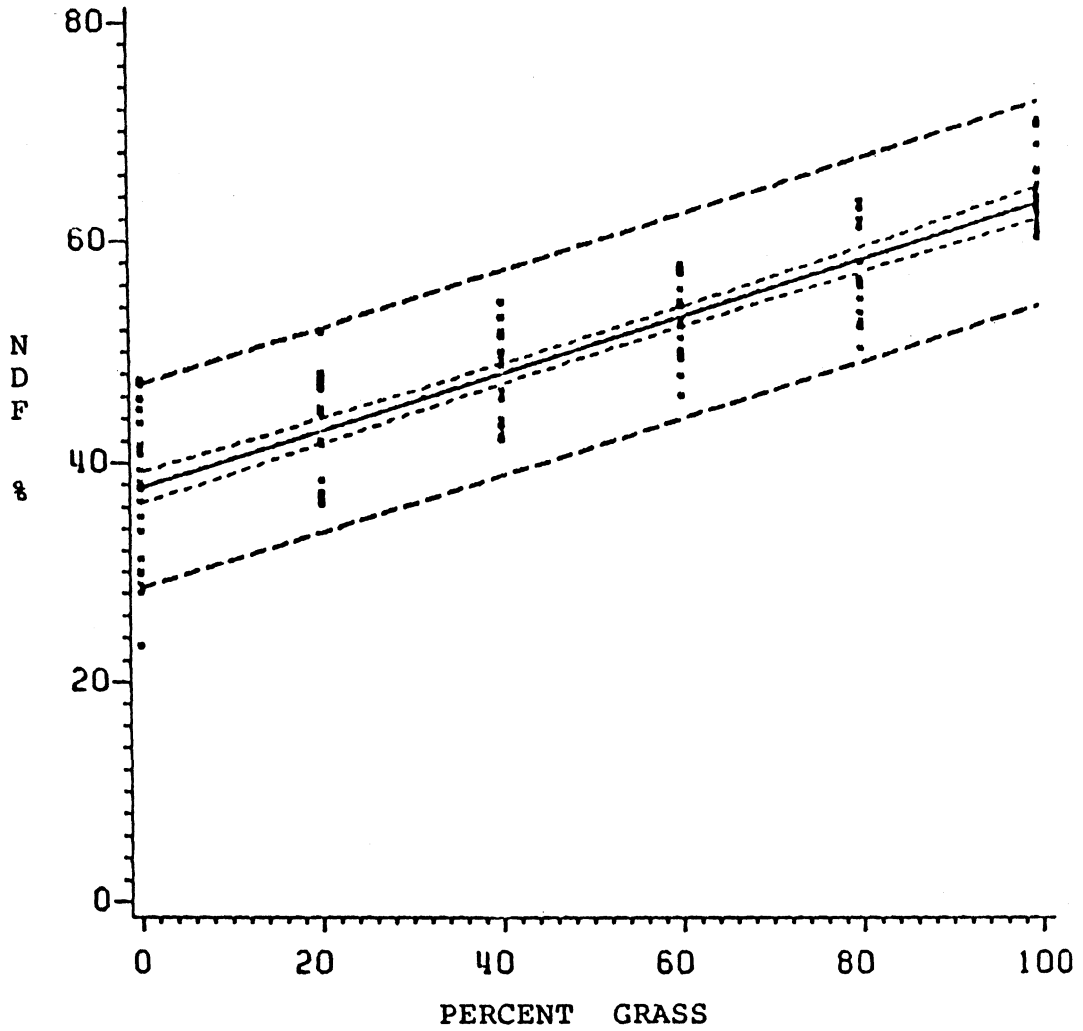

Figure 11: REGRESSION LINE(__), 95% CONFIDENCE LIMITS(--) AND PREDICTION LIMITS(—-) FOR NDF IN FESCUE-ALFALFA MIXTURES (EXP.1)

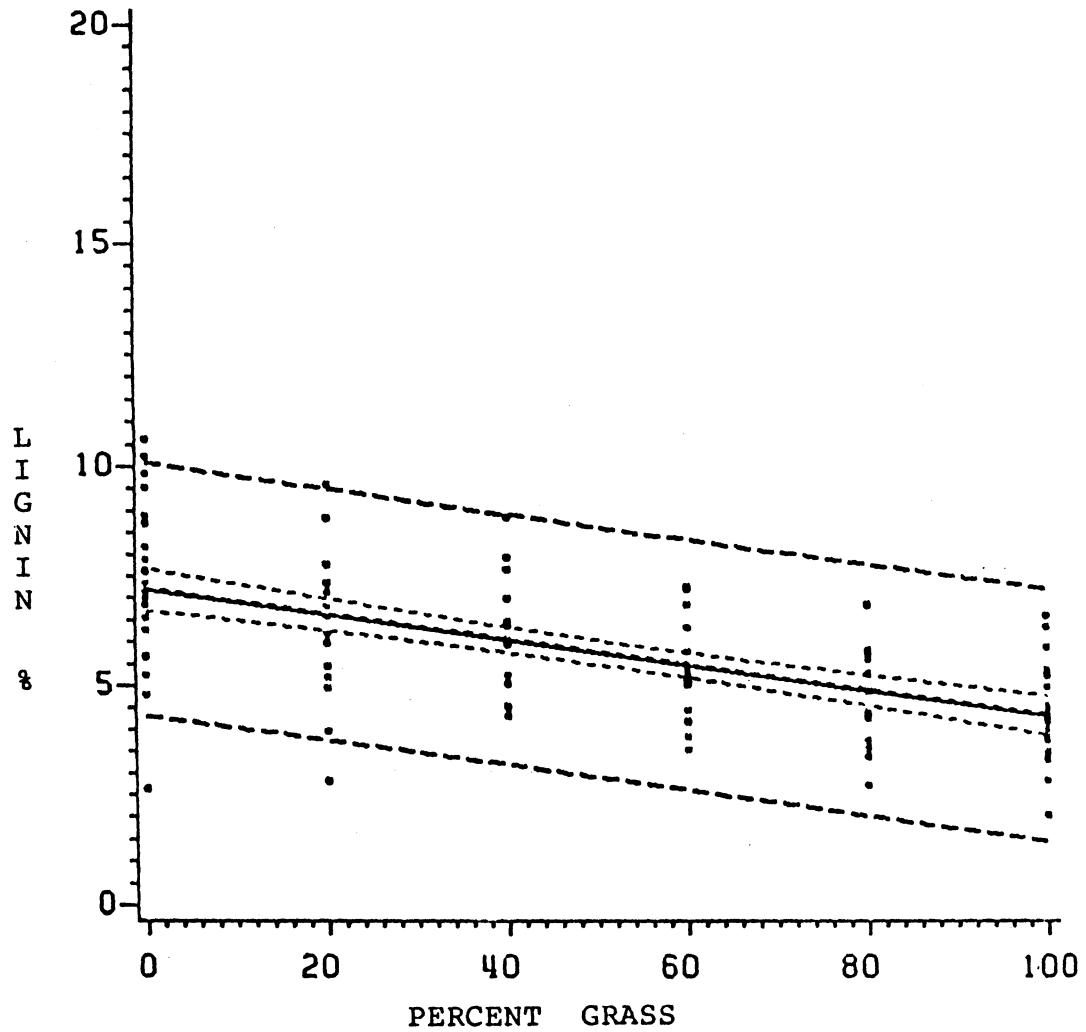

Figure 12: REGRESSION LINE(__), 95% CONFIDENCE LIMITS(--) AND PREDICTION LIMITS(—) FOR LIGNIN IN FESCUE-ALFALFA MIXTURES (EXP.1)

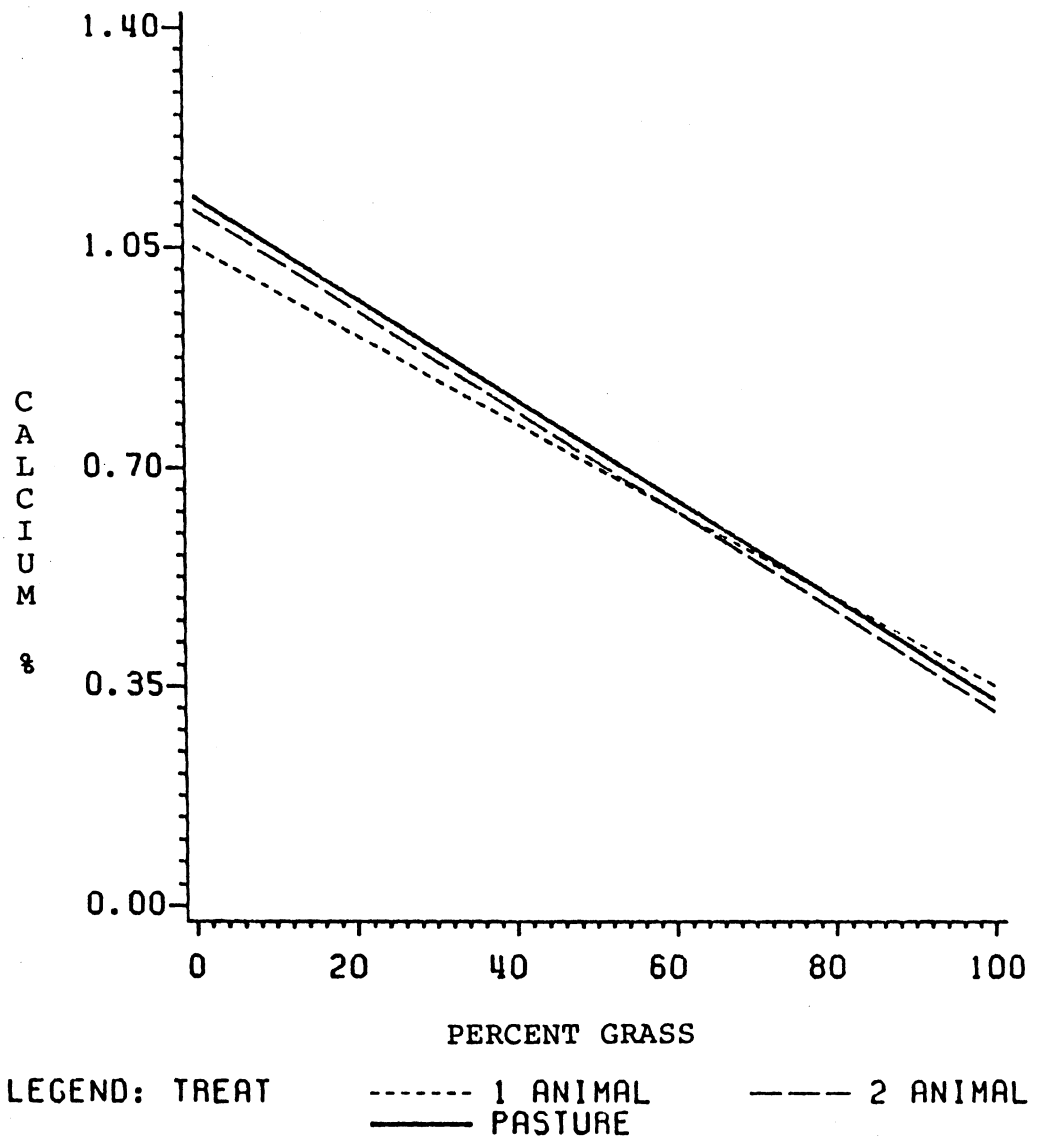

Figure 13: REGRESSION OF CALCIUM CONCENTRATION AND PERCENT GRASS FOR THE PASTURE AND BOTH ANIMALS (EXPERIMENT 2)

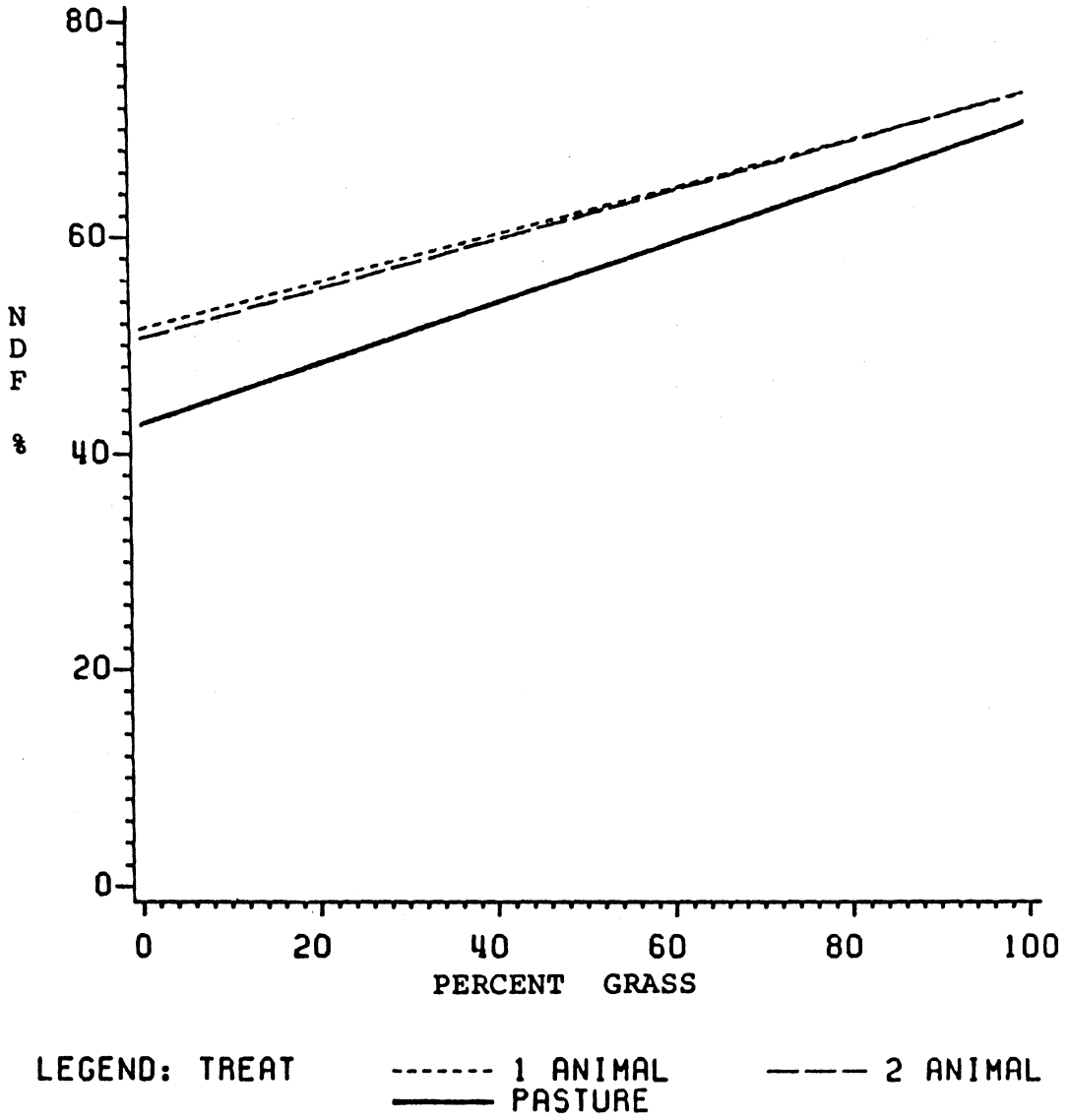

Figure 14: REGRESSION OF NDF CONCENTRATION AND PERCENT GRASS FOR THE PASTURE AND BOTH ANIMALS (EXPERIMENT 2)

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ESTIMATION OF PROPORTIONS OF GRASS AND LEGUME IN EXTRUSA OF ESOPHAGEALLY-FISTULATED ANIMALS

by

Guillermo Pigurina

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Animal Science

(ABSTRACT)

Three studies were conducted to evaluate a method for estimating grass and legume fractions in extrusa samples from esophageally-fistulated animals, based on different concentrations of neutral detergent fiber (NDF), Ca and lignin in grasses and legumes. In experiment 1, NDF, Ca and lignin concentrations were measured in mixtures of six combinations of red clover-orchardgrass and alfalfa-tall fescue (100:0; 80:20; 60:40; 40:60; 20:80; 0:100). The R^2 values were .90, .80 and .22 for Ca, NDF and lignin, respectively. In experiment 2, fresh tall fescue and red clover were collected during 8 d, mixed in the same proportions as in experiment 1, and each fraction was fed to esophageally-fistulated steers. Extrusa samples were collected without loss of saliva. Regression equations developed were $y = 1.08 - .008x$ (R^2=.91) for Ca and $y = 50.9 + .22x$ (R^2=.74) for NDF, where x repre-

sents % grass. In experiment 3, two esophageally-fistulated steers were allowed to graze in 20 4 X 4 m^2 plots of red clover and tall fescue after fasting overnight. The grass/ legume proportions of the extrusas and the grab samples were extrapolated from slopes. The botanical composition of grab samples differed ($P<.001$) from that of extrusas for both components and for both animals. Extrapolation from NDF values tended to overestimate the proportion of grass in extrusa samples and underestimate it in grab samples. Calcium was not affected by salivation and mastication and was more reliable than NDF. Lignin was not an accurate predictor.