ESTIMATING FORAGE MASS OF TALL FESCUE PASTURES AND DRY MATTER INTAKE AND DIGESTIBILITY OF FESCUE FORAGE BY BEEF STEERS

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Estimating forage mass of tall fescue pastures and dry matter intake

and digestibility of fescue forage by beef steers

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ABSTRACT

A series of three experiments were conducted to evaluate different methods of estimating forage mass of tall fescue (*Festuca arundinacea*, Schreb) pastures, and dry matter intake (DMI), fecal output (DMFO) and digestibility (DMD) in beef steers under indoor and grazing conditions. Forage mass was estimated with the herbage cutting method (HCM) and three indirect methods; the pasture capacitance meter (PCM), the plate meter (PPM), and the visual estimation (VE) eight times during 2 yr on six paddocks at the Shenandoah Valley Agricultural Research and Extension Center (SVAREC), Steeles Tavern, VA. Calibration equations were developed at all sampling dates for the indirect methods. Data were analyzed with paddock as the experimental unit, sampling method as treatment, and sampling dates as repeated measures using the mixed procedure of SAS. The r^2 values from the calibration equations pooled across the experimental period (2 yr) were 0.686, 0.751, and 0.862 for the PCM, PPM, and VE, respectively. There were differences ($P < 0.01$) due to sampling method, date, and their interaction. Individual contrasts showed that forage mass obtained by the HCM was overestimated ($P < 0.01$) by the three indirect methods. However, reliability of these subsampling techniques compared with the total harvest of the forage remains to be elucidated.

The second experiment was a digestion trial conducted with confined animals at the Virginia Tech Smithfield Barn with two objectives: 1) to determine the recovery rate (RR) of chromic oxide (Cr_2O_3), and alkanes when dosed to beef steers by means of intraruminal controlled release capsules (CRC), and 2) to evaluate the accuracy of the concurrent estimates of dry matter intake (DMI), fecal output (DMFO), and digestibility (DMD). Six Angus crossbred steers (avg. BW 328 kg ± 31 kg) were allotted to individual

pens with access to water and mineral blocks. Daily diets of tall fescue hay were fed at 1.5% BW, as-fed basis, and were supplied in two equal portions at 0800 and 1700. Seven days before the beginning of the collection period, the steers were dosed orally with Cr_2O_3, and alkanes C_{32}, and C_{36} with CRC. The fecal collection period started on June 25, 2003 and lasted for 7 d. During this time, total feces were collected in special bags twice per day at 0700 and 1600 and a 5% sample was taken from each bag after thoroughly mixing of the feces. Rectal grab samples were also taken at the time of changing the bags. All the fecal samples were placed in plastic bags and kept frozen (-18 °C) pending laboratory analysis. Hay samples representative of each meal were collected in plastic bags and stored at room temperature until analysis. Analytical determinations included DM, ash, CP, NDF, ADF, IVDMD, Cr_2O_3, and alkanes. Data were analyzed using the mixed procedure of SAS. Actual DMI, DMFO, and DMD were 4.74 kg/d, 1.85 kg/d, and 61%, respectively. No difference was found in the RR of either alkanes or Cr_2O_3, during the 7-d collection period. Likewise, actual DMI, DMFO, and DMD were not different (P $\geq$ 0.161) from the estimated values when using the alkanes C_{32}, and C_{36}, or the Cr_2O_3 as external markers. However, estimated values had to be adjusted for the RR of the marker. The use of the pooled samples across the 7-d collection period gave the most reliable estimates of DMFO and DMI, regardless the kind of sample (total collection, grab AM, or grab PM) used in the calculations.

A third experiment was conducted under grazing conditions at the Virginia Tech. Kentland Farm to assess the Cr_2O_3 CRC capsule technique to estimate DMFO and DMI in beef steers. The procedures, in general, were similar to those described above. The main differences were that the steers were heavier (avg. BW 382 $\pm$ 16 kg) and they were grazing a tall fescue pasture instead of being fed. Therefore, estimates of forage mass and quality had to be taken. Results from alkanes were not considered because of a problem with the gas chromatograph. In this trial, it was found that the actual DMFO averaged 3.18 kg/d per steer and the forage IVDMD was 75.83%. Thus, the calculated DMI was 13.21 kg/d. The average Cr_2O_3 RR per steer was extremely high with an average of 1.89. However, when it was used to correct the estimated DMFO and DMI, the values were not different (P $\geq$ 0.846) from the actual ones.

DEDICATIONS

To my parents: Josefina Guerrero Herrera, and Francisco Lopez Guerrero (deceased).

To my wife: Teresa Beatriz Garcia Peniche.

To my children: Tania Beatriz, Isaias, and Teresa Nathalia.

To my brothers and sisters: Gloria, Jorge Adalberto, Francisco Ernesto, Maria Luisa,
Hector Jeronimo, Genaro, y Jose Alfredo.

To all my friends and relatives.

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CHAPTER I
Introduction

Plant and animal breeding programs, diet calculation, and forage quality indices are just a few examples, within the agricultural sciences, in which forage mass, dry matter intake (DMI), and dry matter digestibility (DMD) are basic requirements. On one hand, biomass of grassland vegetation represents the base measurement required for calculation of other grassland attributes that are important in grazing studies, e.g., amount of green vs. dead material, botanical composition, total amount of available nutrients, and the effect of management practices on pastures. The estimates of crop growth rate, and forage production, utilization, and degradation also require estimates of the forage mass before and after a period of time. On the other hand, DMI and DMD probably represent the most important and useful estimates of the forage nutritive value and quality when animal production cannot be determined. Thus, they are necessary in developing forage quality indices such as "the relative feed value" and "the relative forage quality" (Moore and Undersander, 2002). Unfortunately, in trials under grazing conditions forage mass is difficult to measure because the crop cannot be harvested without affecting both animal and pasture performance. Likewise, the actual feed intake and nutritive value of the forage consumed are also unknown. Due to the importance of these traits in making management decisions and explaining results from experiments, managers and researchers usually have to rely on one or more estimating methods.

In pasture sampling, it is commonly accepted that the herbage cutting method (HCM) furnishes a reliable estimate of forage mass, provided an adequate number of representative quadrats or strips have been cut. Mature pastures often present a large variation, requiring a big sample size in order to get a reliable estimate of herbage mass. Direct cut sampling requires high inputs of time, labor, and equipment and may influence production and composition of forage as well as grazing behavior. Therefore, managers and researchers may make the wrong decision of cutting an insufficient number of samples, resulting in inaccurate estimates of forage mass. There are, however, some

indirect methods such as the pasture capacitance meter (PCM), the pasture plate meter (PPM) and the visual estimation (VE) that use the benefits of the double sampling techniques to estimate the pasture DM mass. These methods are easier, cheaper, and faster than the HCM but information is still needed concerning their behavior under different conditions of pasture types and management.

In a conventional indoor digestion trial, both DMI and dry matter fecal output (DMFO) can be measured directly and apparent digestion coefficients are easy to calculate. However, total fecal collection is not always possible, and in grazing trials, for example, neither the animal intake nor the digestibility of the forage eaten can be measured directly. Under these conditions, the use of markers represents the most widely accepted method to estimate the animal voluntary feed intake and the forage digestibility (Van Soest, 1994). Nutritional markers have been classified as internal or external depending upon the natural occurrence of the substance in the feed (Kotb and Luckey, 1972). Examples of internal markers are some chromogens, silicates, acid insoluble ash, lignin, and odd-chain alkanes. External markers include glass beads, charcoal, rare earths, metal oxides, and even-chain alkanes (Marais, 2000). Gelatin capsules, paper tissue, fiber fractions, and other substances have been used as carriers for the markers in digestion trials with cattle, but recently, the use of intraruminal controlled release capsules (CRC) have become a popular method to dose chromic oxide and alkanes. Thus, the objectives for the present study were to:

1. Compare the precision of forage mass estimates of the herbage cutting method with the estimates of three indirect methods; the pasture capacitance meter, the plate meter, and the visual estimation.

2. Determine the recovery rate of chromic oxide and long chain alkanes from controlled release capsules administered to beef steers fed tall fescue hay.

3. Assess the accuracy of the concurrent estimations of dry matter intake, fecal output, and digestibility in an indoor digestion trial.

4. Evaluate the use of chromic oxide controlled release capsule coupled with the in vitro digestibility technique to estimate dry matter fecal output and intake of beef steers grazing a tall fescue pasture.

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CHAPTER II
Review of Literature

Estimating forage mass in grazing trials

Biomass of grassland vegetation refers to above-ground herbaceous material, commonly referred to as dry matter (DM) yield (Mannetje, 2000). Other related terms are forage mass and herbage mass as described by the Forage and Grazing Terminology Committee (1991). Most grasslands used for agricultural purposes are stocked by animals at least part of the time and in many cases year–long (Mannetje, 2000). Researchers and managers of grassland vegetation are interested in knowing the herbage mass because it is the base measurement required for calculation of other important grassland attributes such as amount of green versus dead material, weight of individual species, and total amount of nutrients available from the pasture. Herbage mass is also required to calculate grazing pressure and indicates the effect of different management practices on pastures. Estimates of crop growth rate, and pasture production, utilization and degradation also require the DM yield estimation before and after a period of time.

The problem is that the only way to measure the forage mass of a pasture is by cutting, drying, and weighing the total amount of herbage without any loss of material. That is a difficult and impractical task in grazing trials because usually both pasture and animal performance are being evaluated. Therefore, managers and researchers have to rely on one or more methods to estimate forage mass, commonly using a subsampling technique (Frame, 1981).

When subsampling a pasture, it is accepted that the direct and destructive cutting method furnishes a reliable estimation of herbage mass, provided a correct number of quadrats or strips have been cut (Frame, 1981).Unfortunately, pastures, especially mature grazed pastures, are often variable entities by nature, and a large sample size is required to get a suitable estimation of the single factor of greatest importance, the mass of forage

itself (Wilm et al., 1944). Direct-cut sampling is destructive, requires high inputs of time, labor, and equipment, and may influence production and composition of forage as well as grazing behavior. Destructive sampling also prevents measuring changes of the standing sward in the sampling area. Therefore, researcher may make the wrong decision of cutting an insufficient number of samples, resulting in inaccurate estimates of forage mass (Mannetje, 2000).

Destructive techniques of estimating forage mass

Grassland biomass is defined as the instantaneous measure of the total weight of herbage per unit of land area, preferably measured at ground level (Hodgson, 1979). Therefore, there is only one way to know the actual herbage present at any time in the pasture that is, cutting the sward at ground level and weighing it without any loss of material during the process. This is a difficult task in large scale grazing experiments. Nevertheless, due to the importance of this attribute in making decisions at farm level and explaining experimental results, researchers and pasture managers usually have to cut a subsample of the sward to determine herbage mass (Wilm et al., 1944; Mannetje, 2000)

When subsampling a pasture, clipping furnishes the researchers with an objective index of forage mass, which is accurate, sensitive, and reliable, provided that sampling size is adequate (Frame, 1981). However, in extensive field experiments, estimate of DM yield by clipping may lead to a large number of samples having to be cut because of the natural variability found in grazed swards (Shaw et al., 1976; Vermeiere et al., 2002). The appropriate number of samples depends upon the inherent variability present in the pasture, the magnitude of the treatment differences one wishes to detect, and the level of significance for testing treatment effects (Thomas and Laidlaw, 1981). For example, Michell (1982), analyzing data from the work of Campbell et al., (1962), found that estimations of pasture DM yield made by harvesting strips 0.3 m by 2.7 m had yield coefficient of variation of about 20 %. Assuming a mean herbage mass of 2000 kg of DM per hectare, this indicates a standard deviation of 400 kg of DM per hectare. With this level of variability, in order to detect differences (P = 0.05) of 10% of the mean in

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herbage mass, it would be necessary to harvest about 30 strips per plot. These estimates made by Michell (1982) are in agreement with the results from Waddington and Cooke (1971) who studied the influence of sample size and number on the precision of estimates of herbage production and consumption in two grazing trials. These expeiments were carried out at Melfort, Saskatchewan, Canada and involved four types of pastures in continuous and rotational stocking. The size of the paddocks varied from 0.607 to 2.428 ha and they were sampled for DM yield during two consecutive years using small (0.92 X 0.92 m, 0.85 m^2) and large (0.92 X 2.77 m, 2.55 m^2) cages. Their results showed that when an experiment was replicated two or three times, more than 30 samples were required to estimate a 95% confidence interval around herbage mass. If four replicates were used, 14 to 17 samples were needed for forage production and consumption estimates, respectively. The authors stated that…"clearly the estimation of 95% confidence intervals of seasonal yield was impractical". Under the conditions of these experiments, in order to estimate 90% confidence intervals of production, an average of 19 samples were needed when the experiment was replicated twice, whereas only 10 and 4 samples were needed with three and four replicates, respectively. When comparing small and large cages the results showed a little gain (8%) in precision with the larger cages.

Studies like those of Waddington and Cooke (1971), and Michell (1982) support the idea that sometimes, practical, physical and financial limitations, rather than statistical considerations dictate the number of samples to be taken when estimating forage mass of a pasture. Vermeire et al. (2002) also stated that clipping can provide accurate and precise estimates, but the time and labor required often prevent researchers and managers from collecting an adequate number of samples. Hinkelmann and Kempthorne (1994) insist on the importance for the investigator to have an estimation of the number of replications and samples needed to detect certain minimum difference between treatment effects prior to conducting the experiment.

Other factors to be considered in pasture sampling techniques are the sampling strategy, the sample shape and size, height of cutting, border and observer effects

(McIntyre, 1978; Aiken and Bransby, 1992). In forage evaluation, which usually requires long-lasting trials, it is important to follow the same sampling strategy throughout the study in order to reduce variation among and within sampling dates. There are four main approaches in sampling within plots: simple random, stratified random, cluster, and systematic sampling (McIntyre, 1978). Each of them has a set of requirements to produce a good estimation of the desired variable. In addition, there are methods which use concomitant measures and unbiased selective procedures (McIntyre, 1978). Sometimes, fatigue or a wrong estimate of the sampling time and labor may leads the technician or researcher to the conclusion of taking most of the samples nearby the gates or on an easy-to-sample site of the paddocks, especially when heavy devices such as lawn mower machines are used. This is clearly a mistake that has to be avoided. The use of vehicles facilitates the transportation of the equipment but, it has some disadvantages for the pasture, such as soil compaction, and for the sampling strategy because vehicles cannot be driven at some sites of paddocks located in hilly or swampy conditions or sites with streams, rocks, or excessive trees.

With regard to the shape of the sampling unit or quadrat, it is recognized that the best shape should be the one that maximizes area and minimizes perimeter, which means that the circle would be better than the square and the square better than the rectangle (McIntyre, 1978). In the field it has been found that the rectangular quadrats have lower variations than the square ones, especially when there is a gradient of production or the pasture has been sown in rows and the quadrat is oriented across the rows (Mannetje, 2000). Another accepted fact is that the sample unit should be greater than the size of the biggest plant size involved in the study (McIntyre, 1978). The larger the quadrat the better chance it has to capture the variation among plants within the plot. Nevertheless, there is a compromise between size of the sampling units and the number of samples to be taken. The more numerous the quadrats for the same sampled area, generally, the more precise will be the sample mean.

Another important aspect to be considered when sampling pastures for DM yield measurement or estimation is the height at which the plants have to be cut in order to get

meaningful values (Hodgson, 1979). The cutting height plays an important role in DM yield due to the structure commonly found in pasture plant communities. In general, there is an increase in bulk density from the top to the bottom of the canopy (Lopez, 1995; Prache and Peyraud, 2001). Therefore, a small variation in cutting height will produce a relatively large variation in DM yield. Hodgson (1979) advised to cut at a ground level, but another advice is to cut the forage at a height that can give meaningful estimations of DM yield according to the type of grassland, type of animals and objectives of the experiment (Thomas and Laidlaw, 1981). Thus, cutting height should be set below a minimum grazing height and usually ranges from 1 cm in closely grazed pastures to 10-20 cm in tall swards (Mannetje, 2000).

Non-destructive techniques of estimating forage mass

Non-destructive techniques are also referred to as indirect or short-cut methods (Frame, 1981). They include the double sampling concept as described by Wilm et al. (1944). As the name suggests, this technique involves the sampling of any population by two methods, one which yields data directly on any desired factor such as forage mass, but often is laborious and expensive, and another (short-cut method) which yields data on some factor that is highly correlated with the desired factor and is easier, faster, and cheaper than the former one. The use of any short-cut method in the sampling of forage mass causes a loss in precision per sample unit, as it can provide only an approximation of the actual weight on the factor observed (Wilm et al., 1944). However, since this method is cheaper to apply in the field, many more sample observations may be taken per unit of land area and thus a net gain in precision or efficiency may result. The amount of gain, if any, depends on the relative cost in terms of time, labor, and equipment needed for the double-sampling technique as compared to clipping or mowing for the destructive or direct method. The other and probably most important factor to take into account when using a non-destructive method is the relative accuracy with which forage mass can be calculated from the regression.

In the field of forage sampling, a number of randomized observations are taken by some short-cut method, and in addition, the forage is actually clipped at a relatively small number of sampling points; thus, we have two sets of data available: a large sample containing only observations taken by the short-cut method, and within the large sample, a small sample also containing the actual weights of forage clipped at a portion of the sampling points observed in the large sample (Wilm et al., 1944). Using only the small sample data, a regression is calculated to show the relation of actual forage weight (Y) to the factor observed by the short-cut method (X). In itself, this regression adds nothing to the information on forage weight provided by the small sample alone. However, a relatively precise estimate of forage mass is achieved when the regression equation, also called the calibration equation, is applied and solved for the large set of observations.

Methods of estimating herbage mass indirectly which minimize time, labor and physical removal of herbage have been developed over the years. Some of them include visual estimation (Haydock and Shaw, 1975; Waite, 1994; Smith et al., 2001), plant cover (Anderson and Kothmann, 1982), canopy volume (Thorne, et al., 2002), pasture canopy height (Hutchinson, 1991; Murphy et al., 1995), the use of the pasture plate meter (Bransby et al., 1977; Harmoney et al., 1997; Correll et al., 2003), the single-probe pasture capacitance meter (Vickery et al., 1980; Sanderson et al., 2001), visual obstruction (Laca et al., 1989; Vermeire et al, 2002), and more recently the imaging spectroscopy (Schut and Ketelaars, 2003). Frame (1981) considered the following as the most important reasons for using the double sampling techniques:

a) To reduce the labor, equipment, time or resources needed, and hence the cost of pasture DM yield measurement.

b) To make measurements on large fields or plots (particularly under grazing management), or on remote sites, where it would be impossible to sample swards adequately by cutting techniques.

c) To use in small-scale grazing trials where sampling by cutting could affect a relatively large proportion of the treatment area.

d) To rank treatments in trials with large comparative differences.

e) To provide a guide to the estimation of herbage mass in animal production systems where an absolute measure may not be necessary.

Although non-destructive methods are less accurate in a per sample unit basis than cutting methods, they take less time per observation and involve less physical effort by the operator. Thus, when compared with destructive techniques, DM yields may be estimated more accurately with the indirect methods. According to Mannetje (2000), these non-destructive or indirect methods can be grouped into three categories as follows:

i. Visual estimation,

ii. Height and density measurements, and

iii. Measurements of non–vegetative attributes that can be related to DM yield.

Visual estimations (VE). In practice, the DM yield is normally estimated by visual inspection, a very subjective and inaccurate method. Although experienced operators who are very familiar with the type of pasture under consideration may be able to estimate the DM present in a field within 1 t per ha without any calibration cuts, the procedure is of limited value in serious research (Gabriels and Van den Berg, 1993).

The accuracy of the technique has been improved by making increased use of reference standards in the field, and probably the modification most widely used is the one proposed by Haydock and Shaw (1975). The first step of this procedure is to select either five or nine reference quadrats, which constitute the yield scale against which the yields of sample quadrats are rated. To construct a five- point scale, two quadrats (standards 1 and 5) are placed on low and high yielding areas such that rarely will the DM yield of a sample quadrat lie outside this range. The observers then select a position for standard 3 estimated to have a DM yield half-way between 1 and 5. Then, they select standards 2 and 4, which have yields half-way between 1 and 3, and 3 and 5, respectively. A quadrat frame should be left in position at each standard sample unit and a protection cage if needed in grazed paddocks.

In the second step, the observer team is standardized. After establishing the scale, an initial training period is required in which all observers simultaneously rate a series of quadrats until an acceptable degree of uniformity is achieved (Haydock and Shaw, 1975). The aim is to reach a point where the estimates do not differ by more than 0.25 of a scale unit. During sampling it is wise to return to the standards from time to time to refresh the memory. The standards should also be re-examined after any break in sampling, e.g. after lunch, and each morning where sampling extends over a number of days.

In the next step, the observers walk throughout the pasture in a predetermined pattern and at the same time they rank several observational sampling units or quadrats (usually in a systematic procedure) by comparisons with the five reference standard units. After all the plots or paddocks of the same pasture have been rated, the reference quadrats are harvested and the regression equation of DM yield on the rating scale is calculated. Finally, the yield estimate for any sample is obtained by substituting the rating of that sample in the regression equation generated with the five (or 9) standard units in which, the actual DM yield is regressed on the scores of the indirect method.

When using this calibration type, the observers should check all estimates periodically to maintain uniformity. If there is disagreement all observers should go back to check the standard sample units again. Also, as an alternative, the herbage mass of the standard and observational quadrats can be estimated directly as kilograms per hectare, following the same procedure for calibration. In this way, different paddocks of the same pasture type can be sampled the same date and the standard quadrats can be pooled in order to get a more valid calibration equation that can be applied on paddock of different standing crop.

With the comparative yield method, it is possible to make a large number of yield estimates in a relatively short time in large experiments, a sampling rate of 400 estimates per man per day being possible (Haydock and Shaw, 1975). This method has been used widely alone or in combination with the Dry-Weight-Rank method (Mannetje and Haydock, 1963) or direct estimation of percentages of cover, which improves efficiency

and accuracy. Nevertheless, the VE is a subjective method; the results may be different among observers as it has been pointed out by Aiken and Bransby (1992).

The pasture plate meter (PPM). The principle of this method is that the standing herbaceous biomass of an area of grassland might be related to the density and height of its individual components (Mannetje, 2000). Height and density measurements of a sward can be integrated using a PPM of which, there are many types in use. These devices are given a variety of names, e.g., weighted disc, rising plate, dropping disc, or pasture disc. In general, they consist of a rigid disk or plate, commonly 0.3 to 0.8 m in diameter, with a center hole that accommodates a rod marked in increments along its length. A sleeve attached to the center of the disk, loosely surrounding the center rod, allows the disk to slide perpendicularly along the rod. Disk resting height is observed at a standard time interval after the disk is lowered onto a sward from a specified height. Alternatively, one rising plate initially located at the bottom end of a center rod is supported by the canopy while the center rod is pushed until it contacts ground. Cumulative plate height and observation numbers are manually or automatically recorded on counters, allowing very rapid collection of data (Sharrow, 1984; Griggs and Stringer, 1988).

Bransby et al. (1977) evaluated the effect of four different sizes (0.2 m^2, 0.4 m^2, 0.6 m^2, and 0.8 m^2) of a metal disk of constant weight per unit of area (5 kg/m^2) on DM yield estimations of a tall fescue pasture grazed by yearling steers. In this trial, conducted at Columbus, MO from June to December, the researchers developed eight calibration equations with 50 paired samples for each of the disk sizes. The results showed correlation coefficients (r) for bulk height and DM yield that ranged from 0.79 to 0.94, and all were significant at the 1% level of probability. Neither size nor weight of disk had any significant effect on the residual standard deviation or the r, although the larger disk and the change from reproductive (in June) to vegetative (in Sept.) phase of the pasture altered the regression coefficient. Thus, they concluded that the use of disk meter in tall fescue pastures gave relatively accurate estimates of pasture DM yield in situ with minimum labor requirement. Thus, calibrations based on 50 paired observations should provide an optimum balance between precision and time required to calibrate the disk

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meter when estimating DM yield of tall fescue pastures varying in size from 0.80 ha to 1.60 ha.

Following that study, Vartha and Matches (1977) conducted an experiment with the main objective of reducing the number of harvested samples yet maintaining the required levels of precision. In this trial, the chosen disk meter size and weight were of 0.2 m^2 and 2.5 kg, respectively. They sampled the tall fescue pasture every 5 d from May to October, 1975, taking 25 disk meter readings of pasture bulk-height. They only cut 10 quadrats of 0.25 m^2 in area around the average bulk-height previously determined. The correlation coefficients between herbage mean bulk-height and DM yield of this trial were slightly lower than those from Bransby et al. (1977) and changed by season of the year with values of 0.714, 0.822, and 0.709 for spring, summer and fall, respectively.

Douglas and Crawford (1994), working with pastures of perennial ryegrass on a clay loam soil near Edinburg, Scotland, found that disk estimates were most accurate (r^2 ≥ 0.83) when the herbage mass ranged from 4 to 5 t/ha and when the pasture was in a vegetative stage. Similar results were found by Michell and Large (1983), working with ryegrass pastures. These reports are warning for potential problems of the PPM in estimating relatively small or large biomasses as well as herbage mass estimations from stemmy pastures commonly found when the grass species are changing from vegetative to reproductive stage. Recently, Correll et al. (2003) used the PPM technique to study spatial and temporal dynamics of sward structure at low socking densities in The Czech Republic. Plots of 0.35 ha were sampled weekly or twice per week during the grazing season, May to October, 2000. A calibration equation was built at each sampling date using 60 double sample points, each point covering an area of 0.071 m^2. They found that r^2 values were always above 0.90 and the coefficient of variation of the standard error varied from 0.19 to 0.32. Therefore, they concluded that the PPM was suitable in describing a detailed picture of the sward structure under different grazing treatments.

Different shapes and sizes of the PPM have been used and made with different types of construction material. The calibration equations have usually been developed

from 10 to 50 paired samples of actual DM on PPM reading per plot, dropping the PPM from 0.8 to 1.5 m (Michell and Large, 1983; Sharrow, 1984; Rayburn and Rayburn, 1998). The procedures to standardize the calibration equations (Haydock and Shaw, 1975; Vartha and Matches, 1977) have not been used regularly.

Some authors have found consistently high correlation coefficients ($r \geq 0.80$) between DM yield and PPM readings with calibrations equations fairly constant for extended periods of at least one climatic season (Bransby et al., 1977; Douglas and Crowford, 1994; Correll, 2003). Some others have not attained this goal (Karl and Nicholson, 1987; Aiken and Bransby, 1992; Rayburn and Rayburn, 1998). Differences among these studies can be attributed to different factors, perhaps the most important are the type of pastures and stage of maturity, the canopy height, the forage mass, time of the year, number of samples used for the calibration equation and an observer effect. However, researchers that have used this double sample technique correctly have concluded that simplicity, rapidity, and low cost of the PPM make it a method suitable for use in both large and small scale field experiments. Most of them accept that PPM is a useful, cheap and rapid method in herbage mass estimation from grazed pastures.

The pasture capacitance meter (PCM). Capacitance meters have been used since 1956 and although improved versions have been developed, their performance still leaves much to be desired (Mannetje, 2000). The first versions of the PCM were very heavy, made up of two vertical plates or as many as 50 rods that had to be carried by two men, and their operation was too complicated. With time this electronic device has been improved (Campbell, 1962; Angelone et al., 1980a, 1980b). Vickery et al. (1980) claimed that their improved capacitance meter consists of a single rod probe that weighs only about 1.4 kg, has a digital display, is easy to operate, and is less sensitive to variation in temperature and moisture content of the sward.

The operation of the current pasture probe was described by Gabriels and Van Den Berg (1993) as follows: An oscillator in an electrical circuit produces a signal with a certain frequency. The frequency of the signal is influenced by a capacitor in the

electrical circuit. A change in capacitance of the capacitor results in a change in frequency. The capacitance of the capacitor depends on the dimension of the two plates, the distance between the plates and the type of insulator (dielectric) between them. The aluminum pipe is one plate of the capacitor and the grass acts as the second plate. The air is the dielectric. The grass is in electrical contact with the meter through the ground and the aluminum pipe. The signal frequency of the oscillator in the atmosphere is the reference signal. In the grass, where the capacitance of the capacitor is higher than in the air, the frequency is lower. The difference between the two frequencies is detected by an indicator, can be read on a display, and is called the corrected meter reading. This reading can be converted to DM yield units (kg/ha) by means of a calibration equation of DM yield on readings under the specific working conditions.

Results from different trials in determining herbage mass using the PCM are highly variable. For example, Griebenow et al. (1998) working with an "Alistar George Pasture Gauge Jr." in a mixed temperate pasture (60% grasses-40% legumes) found that the gauge predicted the dry matter determined by the clip method with an r^2 of 0.59 and a coefficient of variation of 31%. It means that the method did not accurately predict the DM yield within a quadrat. Likewise, Gabriels and Van den Berg (1993) compared a pasture probe and two pasture disk meters (plastic or metal) against the cutting of the quadrats. They found that the regression analysis showed a non-linear relationship and residual variances that were approximately proportional to the mean DM yield. They also found large coefficients of variation (CV ≥ 26%) and, concluded that at present it is impossible to make an accurate prediction of the DM yield on the basis of these non-destructive measurements.

A common characteristic of these experiments as well as those from Jones and Haydock (1970) and Sanderson et al. (2001) is that the calibration equation was built over different environments, types of pastures, and sampling dates. Moreover, there are reports in which the authors (Nagy et al., 2001; Sanderson et al., 2001) apparently did not develop their own calibration equation, but used the one given with the instrument for other conditions. On the other hand, with a better control of the sources of variation and

especially when the procedure using the PCM has been carried out for each type of pasture, the results have been good enough to take grazing management decisions on farms (Murphy et al., 1995). Vickery et al. (1980), Michell and Large (1983) and Stockdale and Kelly (1984) supported this conclusion because in their trials the calibration equations were acceptable with good standard error, high correlation coefficients ($r \geq 0.80$) and low coefficients of variation (CV $\leq$ 17 %).

Comparisons between methods. Michalk and Herbert (1977) conducted an experiment to compare four double-sampling techniques with the direct hand clipping in estimating DM yield in low density alfalfa-based pastures at Trangie, NSW, Australia. The indirect methods assessed were 1) pasture height, 2) ground cover, 3) combination of 1 and 2, and 4) PPM. Data were collected once in September 1974 from 57 plots. Each plot was sampled at random and independently with each of the five methods but sampling intensity varied. For hand clipping only five $1m^2$ quadrats were used whereas in the four indirect methods 10 $1m^2$ double samples were used to build the regressions. The results indicate that all the double sampling techniques had a linear relationship and that all of them, except the ground cover, had good and significant correlations with the hand clipping method. However, the PPM proved the most sensitive distinguishing yield differences with a least significant difference 42% lower than that observed by clipping. The PPM was also the most efficient in terms of time.

Harmoney et al. (1997) compared DM yield estimations with four indirect methods on pastures in four farms of Iowa. The methods were the leaf area index, canopy height, a modified Robel pole to measure sight obstruction, and the PPM. Data were collected from four quadrats of 0.21 m^2 in area. They found that the accuracy of these methods to predict forage biomass was dependent on the forage type and species. For the combination of all observations, the modified Robel Pole provided the highest correlations ($r^2 = 0.63$), followed by the rising plate meter ($r^2 = 0.59$) and the canopy height stick ($r^2 = 0.55$). A similar trend was found for only legume species with r^2 of 0.76 and 0.72 for the pole and the PPM, respectively. For warm-season species r^2 were 0.63 and 0.61 for PPM and the Robel pole. But, for cool-season grasses the pole had the

highest r^2 of 0.74, followed by the canopy height ($r^2 = 0.70$), with very low r^2 of 0.40 for the PPM. However, the PPM had the best r^2 (0.85) in only tall fescue pastures. The leaf area index analyzer was the only method not useful under the conditions of the study.

Michell and Large (1983) compared a PPM and a single probe PCM to estimate DM yield from perennial ryegrass pastures at the Grassland Research Institute, Hurley, UK. Their results showed r-values always above 0.90 ($r^2 > 0.81$) without significant differences in DM yield estimations between methods. Likewise, Stockdale and Kelly (1984) compared these two indirect methods on irrigated pastures grazed by dairy cows at Victoria, Australia. The methods were tested before and after grazing and the two of them gave good and similar results with correlation coefficients greater than 0.83 ($r^2 > 0.67$) and coefficients of variation for the rising plate meter and the single probe capacitance meter of 12.7% and 13.3% before grazing and 21.8% and 15.4% after grazing, respectively. Murphy et al. (1995) used the PCM, the PPM, and the sward stick on pastures dominated by Kentucky blue grass and white clover during the grazing seasons of 1989 and 1990. Correlation coefficients and scatterplots in this trial indicated that the three methods provided a DM yield estimation that was related similarly with that of the cut quadrats. However, the degree of association was lower ($r < 0.73$) than the one observed by Stockdale and Kelly (1984), especially for the post-grazing measurements. Anyway, the authors concluded that non-destructive techniques used in this trial provided an easy and quick herbage mass estimate at a level of precision that was adequate for making grazing management decisions on farms.

Gabriels and Van Den Berg (1993) stated that at present it is impossible to make an accurate prediction of the dry matter yield of a certain site on the basis of the non-destructive measurements. In their work to calibrate two indirect techniques (PCM and PPM) to estimate herbage mass from perennial ryegrass pastures in The Netherlands, their regression analysis showed a non-linear relationship and residual variances that were approximately proportional to the mean dry matter. Although, their regressions had good correlations ($r^2 > 0.76$), the lowest possible coefficients of variation were 26.2% and 26.1% for the data set with the metal and the plastic disk, respectively. More recently,

Sanderson et al. (2001) estimated the DM yield of several kinds of pastures on farms in the northeastern region of the United States using a commercial single probe capacitance meter, a rising plate meter, and a pasture ruler. They obtained very low r^2 (0.31) with large residual standard deviations; and concluded that the indirect methods were neither accurate nor precise. Nevertheless, a criticism for these reports is that the authors probably were looking for a calibration equation valid for a large region. They included several sources of variation in the model. For example, Sanderson et al. (2001), included data from four farms (Dairy and beef), three states (PN, MA, WV), several pastures, and sampling dates. Also, it looks as if they did not develop calibrations equations under the local conditions of the experiment, but used the regressions given by the manufacturer of the instrument for pasture conditions in New Zealand. The same kind of mistake was made by Nagy (2001) leading to the conclusion that the PCM was not accurate for the Hungarian pasture conditions.

Dry matter intake and digestibility in feed evaluation

Forage quality and nutritive value are terms frequently used in the literature for feedstuff evaluation. Nevertheless, the precise definitions of the terms have varied in scientific literature and sometimes they have been used interchangeably. For example, Mott and Moore (1985) considered that forage quality was a function of forage nutritive value and voluntary intake. In this scheme, forage nutritive value depends upon its chemical composition, its digestibility and the nature of the digested products while voluntary intake is an expression of the forage accessibility, acceptability and its mean retention time. For them (Mott and Moore, 1985), forage quality, genetic animal potential and feed supplements, as well as environmental factors, would determine output per animal under grazing conditions.

According to Buxton and Mertens (1995), forage quality is a function of the forage nutrient concentration, its intake or rate of consumption, the digestibility of the forage consumed, and the partitioning of metabolized products within the animal. Thus, the forage quality can be expressed as the relative performance of animals when

consuming herbage *ad libitum* and it is often estimated by *in vitro* or chemical means because of limitations in cost and time in using animal trials (Van Soest, 1994).

In other schemes (Beever et al., 2000), the forage nutritive value is defined as its capacity to promote animal production. Forage nutritive value is thought to have three main components: the amount of forage the animal will eat (voluntary intake), the concentration of nutrients in the forage (nutrient content), and the ability of the animal to absorb and utilize the nutrients (nutrient availability). The nutrient availability is mainly dependent on forage digestibility. The nutritive value of a feed is a way to express its ability to provide the required nutrients for animal functions such as maintenance, growth, pregnancy, and milk production.

When production per animal cannot be measured, voluntary intake and DM digestibility (DMD) are the most useful estimates of forage quality (Sollenberger and Cherney, 1995). Buxton and Martens (1995) estimated that of the total variation in animal energy intake from different types of forages, approximately 65-75% may be related to intake, 20-30% to differences in digestibility, and only 5-15% to differences in metabolic efficiency. Based on these features, several indices to estimate the relative quality of the forages have been proposed, among others, the forage quality index, the relative feed value index, and the relative forage quality index (Moore and Undersander, 2002).

Digestibility refers to the portion of a given feedstuff or diet which disappears during its passage through the gastrointestinal tract, thus implying that the absorption process is also involved in this assessment of nutritive value (Merchen, 1988). In a conventional digestion trial, both animal intake and fecal output can be measured accurately and digestion coefficients can be calculated not only for the DM, but also for other components if the content of these can be determined in the diet and feces (Schneider and Flatt, 1975). When digestibility is determined by the simple difference between DM consumed and DM excreted, the term refers to the in vivo apparent digestibility (Merchen, 1988; Rymer, 2000) and can be calculated by:

DMD = [(DMI- DMFO) / DMI] X 100

where:

DMD = Apparent dry matter digestibility (%)

DMI = Dry matter intake (g/d DM basis)

DMFO = Dry matter fecal output (g/d DM basis)

when corrections are made for metabolic (microbial and endogenous) components in the feces, the term refers to the true digestibility, calculated by:

TDMD = {[DMI - (DMFO - M)] / DMI} X 100

where:

TDMD = True dry matter digestibility (%)

M = Metabolic products (DM, g/d)

For fibrous components of the diet, apparent and true digestibilities are almost the same because the metabolic components are almost free of fibrous material. But for other nutrients such as some minerals, ether extract, and especially protein, apparent digestibility underestimates true digestibility due to the presence of nitrogenous compounds found in feces, not due to dietary origins (Van Soest, 1994).

Voluntary feed intake, on the other hand, refers to the total amount of DM ingested by an animal in a period of time when availability is not a limitation (Forbes, 1995). In trials under confinement conditions, most researchers have accomplished this by allowing 10 to15% more of the food than that actually consumed by the animal in the previous day. Some others offer feed such that the excess remaining is the same for each animal (Minson, 1981). Under grazing conditions, there seems to be a quadratic and asymptotic relationship between forage allowance and DMI of healthy animals (Blaser, 1986; Dougherty and Collins, 2003). Due to the importance of voluntary feed intake on animal performance, a similar quadratic relationship has been observed by Schlegel et al. (2000) between forage allowance and the average daily gain of beef steers.

It has been demonstrated that digestibility of the diet and voluntary feed intake are associated in a positive fashion up to a point in DMD and negatively beyond that point

(Conrad et al., 1964; Kahn and Spedding, 1984). These kinds of observations support the theory that physical factors limit DMI in low quality diets, such as forages, whereas metabolic or physiological mechanisms control intake when high quality diets, such as concentrates, are offered to the ruminant animal (Forbes, 1995). Conrad (1966) reported that, when adjustments have been made for body weight and fecal output, DMI was positively correlated to the DM digestion coefficient below a 67% value and negatively related thereafter for cows of moderate milk production. He also stated that this value is not static and could change under different conditions. In general, the higher the energy requirements, the higher the digestion coefficient above which intake is controlled by metabolic or physiological mechanisms. However, under grazing conditions there are many other factors affecting feed intake, and the animal should be able to compensate not only for changes in the forage nutritive value but also for changes in biomass and structure of the pasture (Lopez, 1995; Prache and Peyraud, 2001). Intake may also be affected by other factors such as those related with social activities, climate and weather conditions, and probably different levels of pollution (Young, 1988).

Methods to estimate feed intake

The daily quantity of DM consumed by an animal is a critical measurement to make nutritional inferences about feeds, feeding strategies, and subsequent animal responses (Burns et al., 1994). An accurate determination of DMI, usually coupled with an estimation of DMD, provides the basis for the applications of ration formulation, predictions of animal performance, comparisons in animal feed use efficiency, and forage nutritive value. Although intake is more important than digestibility in assessing forage quality, progress in understanding the basic factors that affect intake has been hampered by our inability to measure it accurately and to separate the influences of animal and diet on intake under grazing conditions (Mertens, 1994). All of the commonly used methods to estimate DMI have unique advantages and disadvantages with an associated error that varies in magnitude (Burns, 1994). In general, these methods can be classified as direct or indirect but it is worthwhile to remember that the only technique that allows the

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quantification of the feed ingested by the animal is the digestion trial as outlined in the next section.

The conventional indoor digestion trial. Grazing trials that quantify animal performance are the best method to measure forage quality, but high inputs of labor, time and capital limit their use. Alternatively, forage quality can be estimated by determining forage intake and digestibility using confined animals or laboratory methods (Van Soest, 1994). The goal in a conventional digestion trial is to measure accurately the amount of feed consumed and of feces excreted over a given period of time. The procedure has been described comprehensively by Schneider and Flatt (1975), Minson (1981), and Cochran and Galyean (1994) and it can be summarized as follows:

In a conventional digestion trial, experimental animals are fed the test diets for a preliminary period of at least 10 d to ensure that residues of feedstuffs consumed prior to the trial have been eliminated from the digestive tract. Consistent levels of intake and other feeding strategies are established during the preliminary period to aid in avoiding drastic fluctuations in excretion. The preliminary period is followed by a collection period of 5 to 10 d. Feces (and urine, if nutrient balance data are desired) are collected daily and composite samples representative of the total collection period are prepared for laboratory analysis. Feces, and urine in nutrient balance studies, can be collected by housing the animal in crates designed to accomplish that purpose. Feces can also be collected in special bags attached to harnesses fitted to the experimental animals. Dry matter intake is then measured as the difference in DM offered and DM rejected. Apparent DMD can also be calculated by the conventional formula {DMD = [(DMI - DMFO) / DMI] X 100}.

Differences in herbage mass. Sward methods for estimating herbage intake are based on the same principles as for the conventional indoor trial where intake is measure by difference (herbage intake = herbage offered - herbage refused). The herbage mass is estimated at the beginning and at the end of the grazing period. The difference gives an estimate of the apparent quantity of herbage consumed per unit area. The calculated

consumption per unit area is then divided by the number of animal days per unit area in order to get an estimation of the intake per animal per day (Meijs, 1982).

This method, technically known as the herbage disappearance rate, has several drawbacks (Burns, 1994). First, the assumption that the decline in herbage mass is entirely due to the consumption by the experimental animals is often an overestimation because forage can disappear for other reasons such as trampling, consumption by other herbivores, and the natural process of plant maturity, death, and decomposition. Second, growth of the plant can be considerable during the grazing period. This problem in herbage accumulation is especially important when the grazing period is larger than 2 or 3 d and requires the use of exclosure cages when the grazing method is continuous stocking. Third, probably the most serious limitation of this method is the intensive sampling required to provide an adequate estimate of the herbage mass combined with the problem that different techniques may render different estimations in DM yield.

Differences in animal mass. Weighing animals to estimate intake over short periods of time was suggested since the 1930's (Le Du and Penning, 1982). To use this method, animals are fitted with harnesses and dung bags for feces collection and container for urine collection. The animals are weighed and then turn out to graze and any water consumption is measured. After grazing, the animals are weighed again and feces and urine production are also measured as well as insensible losses in other animals. This has obvious limitations in most grazing settings but has been used successfully when ingestive behaviour is of interest and grazing periods can be of limited duration (Burns, et al., 1994). A recent modification of this method, called the animal weight telemetry system, has been described by Horn (1981). This technique is based on the use of pressure transducers attached to the base of the hooves of the animals on test and requires special equipment and computer software but offers potential in studies of animal behavior (Penning and Hooper, 1985).

Grazing behaviour. Another approach, to estimate the voluntary feed intake especially useful under grazing conditions, is through the monitoring of the grazing

behavior of the animals by the mechanistic model depicted by Hodgson (1982). With this approach, the daily consumption of herbage by a grazing animal (I) can be viewed as a product of three variables: the time spent grazing (GT), the rate of biting during grazing (RB), and the herbage intake per bite (IB):

I = (GT) X (RB) X (IB)

Two additional variables can be calculated with this method; a) the total number of grazing bites per day as the product of GT and RB, and b) the rate of herbage intake, the product of RB and IB. According to this model, the most important variable affecting intake appears to be the size of the bite, which in turn depends on the volume per bite (mouth capacity) and the bulk density of the sward, an intrinsic characteristic of the pasture structure. Some authors have shown that the animal has the ability to modify, to a certain extent, the grazing time and the biting rate to compensate for deficiencies in the intake per bite (Sollenberger and Burns, 2001).

This model seems adequate to explain the mechanics of grazing, but the accurate measurement of the variables involved represents a serious limitation for the use of this method in determining voluntary food intake. However, recent advances include the use of a modified chewing meter, originally proposed by Penning (1983). This device has been used with grazing animals and provides total grazing time, rumination time, resting time, number of boli ruminated, total jaw movements, and number of intake bites. Eating chews can be calculated by difference between total jaw movements and eating bites plus ruminating chews (Burns et al., 1994).

Water turnover. This method involves the injection of tritiated water to a number of fasted animals, which are kept on water deprivation when they are released into the pasture (Le Du and Penning, 1982). Subsequent blood samples are taken to measure the decrease in radioactivity and this measurement is related to the total water pool of the animal. Forage samples are also taken to determine water content, and water in the animal is then assumed to come from the forage. Apparently, this is not a common method to estimate DMI.

Empirical estimates. There are at least two approaches to estimate DMI of farm animals by the use of empirical calculations. The first is the use of mathematical equations already developed and available in the literature. Examples of these are the predicting equations for fish, swine, poultry, dairy cattle, beef cattle, and sheep developed by the NRC (1987). Some of these equations may have been modified more recently with the modifications included in the last edition of the nutrient requirement of the species of interest such as beef cattle (NRC, 1996) and dairy cattle (2001). The British version of the equations is given by the AFRC (1993). Other countries have handbooks on this topic and there are feeding guides that provide information on most of the domestic animals.

The second approach of these empirical estimates of DMI refers to the back calculation of the animal energy requirements (Baker, 1982). That is, herbage intake (HI) is calculated from the energy requirements for maintenance (Em) and production of the animals (Ep) and the total requirements equated with herbage of a given energy concentration (Eh) by the formula: HI = Em+Ep / Eh. This method is attractive because in its simplest form only the weighing of animal product, record keeping, and calculations are involved. The precision of the estimate is entirely dependent on the adequacy of energy standards and the ability to measure animal production accurately (Baker, 1982). Empirical estimates may be the most common way to estimate DMI in commercial animal production enterprises, but Burns et al. (1994) mentioned that generally they provide little insight in the basic biology of animal intake and its understanding.

Marker techniques. In grazing conditions, the amount of herbage consumed is the major determinant of herbivore production, yet it is one of the most difficult aspects of forage quality to measure or predict (Buxton and Mertens, 1995). A method that adequately estimates DMI of grazing animals remains essential to fully utilize the value of pasture research but continues to be elusive (Mertens, 1994). Under these conditions, the use of some inert reference substances, known as markers, represents the most widely

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accepted method to estimate both animal voluntary feed intake and forage digestibility (Van Soest, 1994).

Marker, indicator, tracer, and reference substance are terms applied by workers in nutrition and physiology to a number of materials used in the qualitative or quantitative estimations of physiological and nutritional phenomena (Kotb and Luckey, 1972). Nutritional markers are employed not only for estimations of fecal output, intake, and digestibility but also for partitioning digestion in various segments of the alimentary tract and for estimating digesta flow and retention time as well (Faichney, 1993).

Kotb and Luckey (1972) stated that for a substance to qualify as a marker in nutritional studies, are: it should be inert with no toxic, physiological or psychological effects; be neither absorbed nor metabolized within the alimentary tract; be completely recovered from either raw or processed food; have no appreciable bulk; mix intimately with the usual food; remain uniformly distributed in the digesta; have no influence on the alimentary secretion, digestion, absorption, normal mobility of the digestive tract or excretion; have no influence on the microflora of the alimentary tract; have qualities that allow ready, precise quantitative measurements; and have physical and chemical properties which make it discernible throughout the digestive process.

Most of the researchers (Kotb and Luckey, 1972; Ellis et al., 1980; Merchen, 1988; Owens and Hanson, 1992; Marais, 2000) accept that there is no marker that completely fulfills all these features, but complete recovery in feces and ease of measurement have been the characteristics of major concern in the search for the ideal nutritional marker. They have also made a broad classification of these substances as internal and external markers. Internal markers are indigestible materials occurring naturally in feeds, and external markers are materials that have to be added to the diet or administered to the animal. Usually, DMD is estimated with internal markers such as lignin, acid insoluble ash, indigestible ADF, or odd-chained alkanes, whereas external markers such as metal oxides, rare earths, and even-chained alkanes are preferred for the

estimation of DM fecal output (Marais, 2000). In his review, Merchen (1988) presented three scenarios in which nutritional markers are thought to be useful:

A. When feed intake is known but total fecal collection cannot be made**.** In this case, either internal or external markers may be used. Animals are fed diets containing the marker or are dosed orally with the marker at regular intervals. Samples of feces are then taken as they are excreted or they are grabbed directly from the rectum. Fecal samples must be taken at intervals, which are defined relative to time of feeding or marker dosage to avoid bias caused by diurnal variations in marker excretion. Fecal samples are then analyzed for the marker substance and fecal output is computed by:

DMFO = Daily dose of marker (g/d) / Marker concentration in feces (g/g DM)

Apparent digestion coefficients can be calculated with usual formula:

DMD = [(DMI- DMFO) / DMI] X 100

When the marker is not completely recovered in feces, the equation has to be adjusted for the recovery rate (RR) which is calculated by:

RR = Amount of marker in feces (g/d) / Daily dose of marker (g/d)

The RR is a correction factor for incomplete quantification of the marker in the feces. Ideally, any index substance should be totally recovered in the feces in order to demonstrate that it is an inert, non-digestible, non-absorbable marker useful. However, both internal and external markers have shown different degrees of recovery, making it advisable for the nutritionist to collect the total fecal output of at least a few animals in order to have a measurement of the RR.

B. When neither feed intake nor fecal output is known, but an estimate of digestibility is desired. This is a common situation in grazing trials that require an estimate of the in vivo apparent digestion coefficient. In this instance an internal marker must be used and DM or any other nutrient digestibility can be estimated if its concentration can be determined on feeds and feces. The general formula is:

Digestibility of nutrient (%) =

$$100 - \left[100 \; X \; \frac{\% \text{ Marker in feed}}{\% \text{ Marker in feces}} \; X \; \frac{\% \text{ Nutrient in feces}}{\% \text{ Nutrient in feed}} \right]$$

This method is called the ratio technique and in order to have an estimate of only the DM digestibility the formula becomes DMD = [100 - (marker concentration in feed / marker concentration in feces) X 100]. With this method, the total collection of feces is not necessary (Burns et al., 1994; Rymer, 2000). The main problem in this case, as well as in the rest of the laboratory methods to estimate intake and digestion coefficients, is obtaining a representative sample of the material that the grazing animal is actually consuming (Le Du and Penning, 1982). Two basic procedures for sample collection are by hand plucking and by the use of fistulated animals (Cook, 1964; Jones and Lascano, 1992). Both of them have advantages and disadvantages, but the botanical composition and ecological complexity of the grassland, as well as the amount of resources available, should be considered before making a decision about sample collection in grazing trials.

C. When neither feed intake nor fecal output is known and estimates of both of them are required. Again, this is the case in experiments carried out under grazing conditions. In such a situation, DM fecal output is estimated by using an external marker (as presented in section A) and digestibility can be determined through the use of an internal marker (as presented in section B). Then DM intake can be calculated as follows:

DMI (g/d) = DMFO (g/d) / (1 - DMD)

Where DMI, and DMFO as above, and

(1 - DMD) represents the indigestible fraction of the diet.

When this formula is used to estimate DM intake of animals in grazing trials, fecal output can actually be measured fitting the animals with special bags and harnesses whereas forage DM digestibility can be estimated by different methods that in general could be classified as follows:

1. In vivo techniques

Indoor digestion trial (Schneider and Flatt, 1975; Cochran and Galyean, 1994).

In situ technique (Huntington and Givens, 1995; Orskov, 2000).

The marker technique (Merchen, 1988; Mayes et al., 1995; Marais, 2000).

2. In vitro techniques.

Digestion with rumen fluid (Tilley and Terry, 1963; Stern et al., 1997).

Enzymatic methods (Weiss, 1994; Adesogan et al., 2000).

Fermentation end-products (Stern et al., 1997; Williams, 2000).

3. Fecal and forage index techniques.

Fecal nitrogen (Cordoba et al., 1978; Le Du and Penning, 1982).

Near infrared reflectance spectroscopy (Shenk and Westerhaus, 1994).

4. Mathematical models.

Empirical equations (Weiss et al., 1992; Moore and Undersander, 2002).

Theoretical-based equations (Van Soest, 1994).

5. Other laboratory methods estimate DMD using relationships with chemical components of the forage samples, particularly fibrous fractions such as NDF, ADF, and lignin. However, it is good to remember that no individual fiber fraction is closely correlated with DMD over a wide range of forages. Therefore, samples should be selected from locations and species similar to those of the forage to be tested (Coates and Penning, 2000).

Marker dosing methods

All marker procedures use one of two types of dosing methods. The marker can be administered either as a single pulse-dose, or it can be provided constantly or frequently for a period of days in an attempt to reach steady state conditions (Owens and Hanson, 1992). Pulse-dosing typically is used to estimate digesta volume and retention time in specific parts of the gut and, with this information, digesta flow (and excretion) can be calculated. Continuous dosing is used primarily to measure instantaneous flow at a specific point in the digestive tract and to estimate fecal output. The goal of continuous or frequent dosing, or infusion of a marker is to label the digesta uniformly so that the ratio

of digesta (or fecal output) to marker is constant. This permits flow rate (DM/d) to be calculated simply by dividing dosage rate (g/d) by marker concentration (g/g DM). Unfortunately, steady state conditions may never exist under either grazing or pen conditions because of the ingestive behavior, and the consequent irregular concentration of the marker in the ruminal pool.

In grazing trials if the animals are restrained, the handling would be stressful and represent a disruption of the normal grazing and excretion activities (Burns et al., 1994). Even though there are many difficulties with this procedure, an advantage in using daily dosing is that the kinetic properties of the marker do not influence the estimate of fecal output, provided a constant marker flow can be attained in the feces (Van Soest, 1994). Temporal variability can be partially compensated by more frequent collection of samples and more frequent administration of the marker but animal stress, labor, and disruption of normal animal activities increase proportionally (Burns et al., 1994).

Controlled release capsules. The animal stress produced when handing animals for marker dosing and sampling may alter eating behavior, fecal output, and forage intake. Therefore, the controlled release capsule (CRC) technique was developed to overcome the difficulties in once or twice daily dosing with chromic oxide (Cr_2O_3) as well as the diurnal variation in marker concentration in feces (Burns, 1994). Recently, CRC are also used to dose even-chained alkanes as external markers to cattle (Berry et al., 2000) and sheep (Dove et al., 2002). The capsules have been described by Pond (1990) as follows:

"Each capsule consists of a plastic barrel and wings, a stack of tablets containing the fecal marker, a spring and a plunger. The wings are folded and held in place during dosing with water soluble tape. On contact with ruminal fluid the wings unfold to decrease the probability of regurgitating the capsule. The release of the marker starts when water (or ruminal fluid) passes through the end of the capsule and is absorbed by the first tablet. The tablets contain water soluble compounds which form a gel when in contact with moisture. The gel containing the marker is slowly extruded through the end

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of the capsule by the spring-loaded plunger. When steady state is achieved (4 to 6 d after dosing) the mass of the marker released by the capsule is equal to that appearing in the feces. Fecal samples may be collected between d 6 and 20 in cattle and d 5 and 25 in sheep. These capsules have the advantage of one time dosing, allowing flexibility for sampling and reducing amount of labor and animal stress. But to provide reasonable estimates of fecal output the rate of marker release must be constant over a period of time and not affected by diet, animal or level of intake".

In evaluating the usefulness of these devices, results have been contradictory. For example, Parker et al. (1989) working with the captec controlled-release chromic oxide, found uniformity in the CRC plunger travel, almost complete recovery of Cr in feces and low diurnal variation in chromium concentration in feces when the CRC where tested in Romney wether lambs fed alfalfa chopped every day. Nevertheless, wethers with CRC had lower DMI and DMFO than those without CRC (Parker et al., 1991). Brandyberry et al. (1991) tested the efficacy of continuous-infusion pumps and CRC for administering three external markers (Co-EDTA, $YbCl_3$, and Cr_2O_3) to predict fecal output of six ruminally fistulated steers fed chopped alfalfa at 2% of BW. In this trial, the markers reached equilibrium after 100 to 120 h (4.2 to 5 d) after initiating infusion. Thus, concentration in the feces was relatively stable and fecal output estimates obtained from samples taken once daily in the morning or in the afternoon were not different (P > 0.10) but the release rate was only 0.78 of that stated by the manufacturer. Therefore, when they compared the actual DMFO with that obtained by the marker, without the correction due to the incomplete marker recovery, they found that the Cr_2O_3 method overestimated (P < 0.05) the actual value. The same problems of different estimated than predicted values in fecal output and DMI were detected by Buntix et al. (1992) and Luginbuhl et al. (1994), working with sheep, but they did not apply the correction for the low recovery rate of Cr found in their trials. When this was possible, as indicated by Momont et al. (1994), recalculated values were very similar to those attained by total collection. Other problems associated with the use of the Cr_2O_3 capsules are the possible variation in release rate due to diet, level of intake, and animal (Burns, 1994). These results indicate

that total fecal collections on a subset of animals used in the study may be necessary to adjust marker estimates of fecal output as was pointed out by Hatfield et al. (1991).

Milne (2001) stated that the use of n-alkanes as internal and external markers has become the method of choice in this field of the animal ruminant nutrition and CRC have been developed to dose animals with the even-chained alkanes dotriacontane (C_{32}) and hexatriacontane (C_{36}). The accuracy of intake estimation based on the use of alkane CRC and fecal grab sampling on cattle was evaluated with success by Berry et al. (2000). Likewise, Dove et al. (2002) studied the effect of type of diet, level of intake, and frequency of feeding on the accuracy of estimates using the alkane technique in sheep. These trials indicate that intra-ruminal CRC provides a satisfactory means of delivering an accurate, daily dose of alkanes for the estimation of DMFO, DMI, and DMD in ruminants. In general, the opinion with regard to the use of the intra ruminal CRC as a means to dose animals with markers is that they have the potential to reduce time, labor and disruptions of the animal's behavior in grazing studies. However, there is a question about the actual amount of marker that is being released, hence, verification of the marker results by comparisons with total collection procedures is advisable (Hatfield et al., 1991; Burns et al., 1994; Momont et al., 1994; Dove et al., 2002).

Chromic oxide as a marker in digestion trials. Oxides and salts of trivalent and tetravalent metals such as titanium, chromium, cobalt, ruthenium and hafnium have characteristics of inert markers and have been used as such with varying degrees of success (Marais, 2000). The most common of these compounds is chromic oxide (Cr_2O_3), which was introduced as an external marker by Edin in 1918 and since then it has probably been more widely used for estimations of both digestibility and fecal output than any other single substance (Knapka et al., 1967). Chromic oxide is a dense, green powder that tends to travel as a suspension in digesta at a rate independent of that of either the particulate or the liquid phases (Merchen, 1988).

Chromic oxide is more suitable as an external marker to estimate fecal output in digestion trials than in passage rate trials (Van Soest, 1994). If a regular and constant

marker concentration in the total fecal output can be achieved, then Cr_2O_3 can provide a good estimation of the *in vivo* DM digestibility because this measurement depends on even distribution of the Cr_2O_3 in the feed and its constant passage down the digestive tract. Oxide bullets (CRC) that will release the respective element at a constant rate are an alternative that may improve the estimations of DM intake and digestibility and at the same time reduce the inputs in labor and time in grazing trials.

Momont *et al.* (1994) conducted two experiments to evaluate the use of Cr_2O_3 controlled-release boluses and alkaline hydrogen peroxide lignin as external and internal markers for estimating DMFO, DMI, and DMD by sheep and cows consuming mature prairie grass hay.

In experiment 1, a digestion trial was conducted with 20 Hampshire ram lambs allowed *ad libitum* access to mature prairie grass hay and fed one out of four supplements that provided 45 g of CP per head, per day. Lambs were housed in metabolism cages and provided free access to water. The experiment consisted of a 21-d adaptation period followed by two 3-d collection periods. Seven days before the beginning of the collection period, lambs were orally administered Cr_2O_3 intra-ruminal boluses. They found that supplementation of lambs fed mature prairie grass hay did not affect daily Cr_2O_3 excretion rate (P = 0.38) or fecal alkaline hydrogen peroxide lignin recovery (P = 0.47). Predicted versus actual DMFO, intake, and digestibility values were similar (P ≥ 0.20) across supplemental treatment. Dry matter intake of lambs was 2.0% of body weight and mean Cr_2O_3 excretion rate was 224 (±4.4) mg/d, 11.4% higher than the manufacturer's reported value of 201 mg/d. Mean alkaline hydrogen peroxide lignin fecal recovery was 97.8% (± 2.5%).

In experiment 2, the digestion trial was conducted with eight mature cows that had *ad libitum* access to mature prairie grass hay with no supplement. Cows were housed in individual pens and the trial consisted of a 14-d adaptation period and a 5-d collection period. Seven days before the first fecal collection, cows were administered the Cr_2O_3 controlled release boluses. Additionally, on day 4 of the collection period fecal grab

samples were taken at 4-h intervals to evaluate diurnal variation in marker recoveries. The results show that the DMI of cows was 1.6% of BW. The mean Cr_2O_3 excretion rate was 1,662 ($\pm$ 63) mg/d, 11.8% higher than the manufacturer's stated release rate of 1,505 mg/d. Mean alkaline hydrogen peroxide lignin fecal recovery was 95.9 % ($\pm$ 3.2 %). Using paired comparisons, predicted versus actual DMFO, DMI, and DMD values were not different from zero for fecal grab samples or samples from total fecal collection. There was no diurnal variation of Cr_2O_3 or alkaline hydrogen peroxide lignin excretion ($P > 0.69$). Related to the use of Cr_2O_3 controlled release capsules, they concluded that after verification of the manufacturer's release rate, this technique has the potential to reduce animal handling, time, and disruption of animal grazing patterns compared with twice-daily administration of Cr_2O_3 recommended for other methods.

Because of its extensive use, chromic oxide has been taken as the reference marker in some studies. For example, Titgemeyer et al. (2001) compared the accuracy of chromic oxide (Cr_2O_3) and titanium dioxide (TiO_2) in estimating DM digestibilities and fecal outputs of steers fed four grain-based diets. In this trial, steers were adapted to diets for 9 d and total fecal collections were made for 4 d with the use of feces collection bags. The results did not show significant differences among diets in intake ($P = 0.33$) and digestibility ($P = 0.07$). Fecal recovery of Cr_2O_3 and TiO_2 averaged 98.4% and 90.0% across diets, respectively. Estimates of fecal output and digestibility with the use of Cr_2O_3 were not different ($P = 0.30$) from those based on total fecal collections. Titanium dioxide led to greater ($P < 0.01$) estimates of fecal output and lower ($P < 0.01$) estimates of digestibility than either total fecal collections or the use of Cr_2O_3. Based on their results, Titgemeyer et al. (2001) claimed that variation among animals and time of day should not influence digestibility estimates if enough animals are used, an adequate sampling protocol is implemented, and marker recovery average 100%.

In experiments to measure digesta kinetics, flow, and mean retention time throughout the gastrointestinal tract, chromium chelated to ethylenediminetetraacetic acid (Cr-EDTA) is used as a liquid phase marker and chromium mordanted to cell wall constituents (Cr-NDF) as a particulate marker (Marais, 2000). Nevertheless, caution must

be observed whenever Cr-NDF is used as a particulate marker because chromium can be bound firmly to the cell wall constituents. This binding may affect particle passage rate due to the increased density of the feed (Ehle et al., 1984) and can cause a reduction in digestibility of the bound material (Mader et al., 1984).

Udén et al. (1980) conducted a series of experiments to investigate the use of chromium (Cr) and cerium (Ce) mordanted to plant cell walls as particulate markers and Co-EDTA and Cr-EDTA as liquid markers. They mordanted fiber from timothy grass, and alfalfa, and described the methods of marker's preparation and analysis. The tests of mordant stability consisted in one or more of the following methods:

1) Refluxing for 1 h in a 2.9 % solution of sodium lauryl sulphate (pH 7),
2) Refluxing for 1 h in a 1.8 % solution of EDTA (pH 7),
3) Incubation for 12 h at 39 °C in 0.1 M HCl,
4) Incubation for 12 h at 39 °C in 0.01 M HCl, and
5) *In vitro* rumen digestion for 48 h.

The results of the *in vitro* experiments showed that the 0.01 M HCl and the *in vitro* treatment had a negligible effect on the stability of Cr-mordanted fibers. As the concentration of Cr mordanted to the fiber increased, *in vitro* digestibility of the fiber decreased, becoming greater than 96% indigestible at above 8% Cr on the fiber. The recovery of Cr increased when the levels of mordanted Cr increased, reaching 98% of recovery at a Cr concentration of 12%. Data obtained from the *in vivo* experiment confirmed the insignificant absorption of Cr during its passage through the gastro-intestinal tract. From these experiments, the authors suggested that Cr-mordant fiber fulfilled most criteria as a particulate marker.

Bruining and Bosch (1992) studied the effect of Cr-NDF particle size on the estimated rate of passage of particles measured by fecal samples of lactating and non-lactating dairy cows. They found that fractional rates of passage did not differ significantly between lactating and non-lactating cows and averaged 2.0% h^{-1}, 4.1 % h^{-1}, 8.6 % h^{-1} and 14.9 % h^{-1} when calculated from fine Cr-NDF (0.6-1.0 mm), extra fine Cr-

NDF (<0.3 mm), Co-EDTA from feces, and Co-EDTA from rumen, respectively. These results indicate that experiments, in which rates of passage are determined using Cr-mordanted NDF, the particle size of the marked fiber has a great influence on the calculated rates of passage. Perhaps, Cr-NDF is only representative of the fraction of rumen particles with the same size and density as the marker.

Ehle et al. (1984) studied the effect of three chromium concentrations in the mordanted solution (2, 8 or 32 % of the dry cell wall weight of alfalfa) on the particle density and turnover rate of dairy cows. They found that density and Cr concentration of mordanted alfalfa fiber influenced ($P < 0.05$) estimates of particle turnover rate. Another disadvantage of the Cr-mordant is the effect of the exhaustive chemical extraction of cells' contents during preparation of cell walls. This extraction may alter particle size and density, as well as the attack of digestive enzymes (Ellis et al., 1980).

In spite of its critics, chromium is still one of the most common external markers used in nutritional studies. Dry matter intake, digestibility and rate of passage in different species have been estimated by the use of chromium by different researchers (Faichney and White, 1988; Molina et al., 2000). This interest might be due to the fact that total fecal output can be estimated with an adequate level of accuracy using Cr_2O_3 as external marker, if a constant fecal output of the marker can be achieved (Van Soest, 1994). Ahvenjärvi et al. (2001), using Cr-labeled straw and indigestible NDF as markers, evaluated the effect of unrepresentative sampling of digesta upon the accuracy of fiber flow measurements. Their results support the idea that the accuracy of digesta flow measurements should be adequate if the indigestible marker is homogeneously distributed in the digesta particulate matter.

The use of alkanes as nutritional markers. Alkanes are completely saturated aliphatic hydrocarbons (Mayes et al., 1995). Although, minor components, hydrocarbons appear to be ubiquitous to the cuticular wax of higher plants. The predominant hydrocarbons of most plants are n-alkanes, which usually occur as mixtures, ranging in chain length from 21 to 37 carbon atoms. Over 90 per cent of the n-alkanes of most

higher plants have odd numbers of carbon atoms, with nonacosane (C_{29}), hentriacontane (C_{31}), and tritiacontane (C_{33}) usually having the highest concentration in herbage (Dove and Mayes, 1991). The relative simplicity of analysis by gas chromatographic methods and the inertness of these compounds were the primary reasons for considering the use of alkanes as fecal markers (Mayes and Lamb, 1984; Mayes et al., 1995). Initially, they were proposed to estimate diet DMD using a natural odd-chain alkane in the forage as internal marker and the formula:

DMD = [1 - (RRi X (Hi / Fi))] X 100

Where:

DMD = Apparent dry matter digestibility (%)

RRi = Recovery rate of the odd chained (i) alkane used in the formula

Hi = Concentration of the i alkane in the herbage

Fi = Concentration of the i alkane in the feces

Mayes et al. (1986) developed a double alkane procedure for estimating herbage intake of sheep with or without supplementation. In this approach, animals are dosed with known quantities of an even-chained alkane and intake is estimated from the daily dose rate and dietary and fecal concentration of the dosed even-chained alkane and a natural, odd-chained alkane adjacent in chain length. The fecal recovery of the plant derived and orally dosed alkanes is incomplete, but alkanes of adjacent chain length (e.g. C_{32} and C_{33}) have been shown to have similar recovery rates in sheep (Vulich et al., 1991; Dove and Olivan 1998; Dove et al., 2002) and cattle (Unal and Garnsworthy, 1999; Berry et al., 2000). Hence, if such a pair of alkanes is used, the effects of incomplete recovery cancel out each other in the calculation of intake by using the equation:

HI = Dj / [(Fj / Fi) * Hi - Hj]

Where:

HI = herbage intake

Dj = Dosed even-chained alkane

Fj and Fi = fecal concentration of even- (j) and odd-chained (i) alkane

Hj and Hi = herbage concentration of even- (j) and odd-chained (i) alkane.

Since the 1980's the use of alkanes as nutritional markers has gained popularity and it has become the method of choice to estimate DMI in grazing animals (Milne, 2001). According to Mayes and Dove (2000), the double alkane technique to estimate DMI offers advantages over the use of other external markers, such as Cr_2O_3. The technique can give accurate assessments of dietary intake of grazing animals. It allows for between animal variation in diet digestibility and thus provides estimates of individual intakes of group fed animals, which can be useful in genetic evaluations. It can accommodate the feeding of supplements to the animal, provided individual intakes of supplements are known or can be estimated. Gas chromatography analysis allows both plant (internal) and dosed (external) markers to be determined at the same time, which reduces analytical errors and bias due to different laboratory procedures. The method can be extended to measurements of botanical composition of the diet if the alkanes profiles can be determine in the forage and feces.

Nevertheless, the large variation in the content of alkanes found in the forage DM of different plant species (Table 1) prevents researchers to use standards from general application across different experimental conditions (Moshtaghi Nia and Wittenberg, 2002). Although these differences may reflect variation between laboratories in methods of alkane analysis (Lewis et al., 2003), variations in alkanes concentration have been found in different parts of plants or in the same species grown in different locations or cut at different stage of maturity (Dove and Mayes, 1991). Casson et al. (1984) and Laredo et al. (1991) have suggested that in order to get reliable estimates of forage intake, the concentration of the natural alkanes should exceed 50 mg/kg DM. Concentrations of alkanes C_{27}, C_{29}, $C_{31,}$ and C_{33} of some species of temperate forages are shown in Table 1. Other alkanes are not shown in Table 1 because their concentration in the forage DM is too low to be useful as markers and because from all the natural alkanes, C_{31} and C_{33} are the most commonly used in estimating DMI and DMD (Mayes et al., 1995). Concentrations of C_{27}, C_{29}, $C_{31,}$ and C_{33} are below this level for several species of temperate forages (Table 1).

Table 1. Most common odd-chained n-alkane concentrations (mg/kg) in the dry matter of selected temperate forage species (cool season grasses)

Forage species	Alkanes				Reference
	C27	C29	C31	C33	
Bromus catharticus	15	116	60	34	Bugalho et al. (2004)
B. inermis	29	34	76	8	Boadi et al. (2002)
B. riparius	52	77	139	31	Boadi et al. (2002)
Dactylis glomerata	16	23	22	9	Boadi et al. (2002)
Festuca arundinacea	14	80	307	63	Piasentier et al. (1995)
F. arundinacea	7	48	151	43	Ordakowski (1998)
F. arundinacea	12	75	239	76	Boadi et al. (2002)
F. arundinacea	42	129	216	59	Bugalho et al. (2004)
F. pratensis	21	159	298	102	Boadi et al. (2002)
F. rubra	16	23	28	8	Boadi et al. (2002)
Lolium multiflorum	40	230	242	57	Dove and Mayes (1991)
L. perenne	38	63	108	82	Mayes and Lamb (1984)
L. perenne	19	73	137	116	Mayes et al. (1986)
L. perenne	36	142	220	99	Malossini et al. (1990)
L. perenne	26	163	261	110	Laredo et al. (1991)
L. perenne	113	270	256	36	Dove and Olivan (1998)
L. perenne	34	93	181	137	Dove et al. (2002)
Phalaris arundinacea	18	21	11	4	Boadi et al. (2002)
Phleum pratense	24	15	17	14	Dove and Mayes (1991)
Poa pratensis	14	108	286	108	Ordakowski (1998)

However, more important than the alkane content of the forage, and feces as it is reported sometimes, is the recovery rate of the alkanes involved in the fecal ratio (Fj/Fi) in the formula to calculate DMI (Dove and Mayes, 1996). Identical fecal recoveries of herbage (odd-chain) and dosed (even chain) alkanes is a prerequisite for reliable intake estimates (Mayes et al., 1986). Unfortunately, this is not always the case. Frequently, the recovery rate shows a quadratic relationship with the carbon chain length of the ingested and dosed alkanes (Mayes et al., 1986; Casson et al., 1990; Dove et al., 2002) and sometimes there is no a defined relationship (Piasentier et al., 1995; Moshtaghi Nia and Wittenberg, 2002; Hendricksen et al., 2002). So far, there is no a clear explanation of this different behavior of the alkanes, but it has been demonstrated that they disappear selectively from the digesta in different sections of the gastrointestinal tract (Ohajuruka and Palmquist, 1991; Dove and Mayes, 1991). A compilation of some recovery rates found in sheep and cattle are shown in Tables 2, and 3, respectively.

Table 2. Recovery rates (percent) of selected alkanes found in sheep

| Forage species | Alkanes | | | | | Reference |
	C_{31}	C_{32}	C_{33}	C_{35}	C_{36}	
Lolium perenne[a]	83	---	91	97	---	Mayes and Lamb (1984)
L. perenne	85	89	89	93	---	Mayes et al. (1986)
L. perenne	78	86	84	95	92	Dove and Mayes (1991)
L. perenne	93	88	88	91	86	Vulich et al. (1991)
Festuca arundinacea	86	90	83	---	---	Piasentier et al. (1995)
L. perenne[b]	93	96	95	---	99	Dove and Olivan (1998)
L. perenne	80	94	93	103	98	Dove et al. (2002)

[a] Mixed with Trifolium repens

[b] Supplemented with sunflower mill

Table 3. Recovery rates (percent) of selected alkanes in cattle

| | Alkanes | | | | |
Forage species	C_{31}	C_{32}	C_{33}	C_{36}	Reference
Lolium perenne	59	77	81	93	Mayes et al. (1986b)
Mixed of hays	---	95	94	95	Unal and Garnsworthy (1999)
Mix of forages	76	87	85	81	Berry et al. (2000)
Bromus riparius	59	84	63	83	Moshtaghi and Wittenberg (2002)

Some general observations can be drawn from the Tables 1, 2, and 3. First, in cool season grasses, the relationship between the content of odd-chained alkanes and the length of the carbon atom seems to be curvilinear with hentricontane (C_{31}) being the most abundant. Second, the recovery rate does not always follows the same relationship, but usually the dosed C_{32} and the natural C_{33} have the most similar recovery rates. Third, there are more trials conducted with sheep and the results look more consistent with sheep than with cattle.

Dove et al. (2002) evaluated the level and frequency of feeding and the number of intra-ruminal controlled release capsules (CRD) administered to sheep on the release rate of alkanes C_{28} and C_{32}, and the resultant accuracy on estimates of herbage intake and digestibility. They found that a linear alkane release commenced after a lag period of 2 to 3 d, and became stable by 6 to 7 d at mean rates of 40.1 mg/d for C_{28}, and 41.7 mg/d for C_{32}. Those recoveries are close to the target rate of 40 mg/d, stated by the manufacturer. They did not find effects (P > 0.05) of neither the frequency of feeding nor the number of CRD on C_{28} and C_{32} release rate, recovery rate and accurate estimation of intake. There was no effect (P > 0.05) of level of feeding on CRD release rates and the differences (P < 0.001) observed in fecal alkane concentrations were consistent with the difference in

fecal output caused by feeding level. As a result, the accuracy with which known intakes and digestibilities were estimated was not affected by level of feeding. The conclusion of this study was that intra-ruminal CRD provides a satisfactory means of delivering an accurate, daily dose of alkanes for the estimation of herbage intake, fecal output, and digestibility in sheep.

Other authors have used this method with different degrees of success, working with other animal species, such as dairy cows (Unal and Garnsworthy, 1999; Berry et al., 2000), beef cattle (Hendricksen et al., 2002), horses (Ordakowski et al., 2001; Stevens et al., 2002), and giraffes (Hatt et al., 1998). Most of them have concluded that n-alkanes have good potential as markers for evaluating the forage nutritive value of herbivores.

CHAPTER III

Manuscript 1. Comparisons among four methods for biomass estimation of tall fescue pastures

ABSTRACT. In forage evaluation and grazing studies, forage mass is a basic measurement required in calculating other important grassland attributes such as growth, utilization, and decomposition. The problem is that forage mass is difficult to measure in large scale grazing trials because usually total harvest is not possible. In this situation, researchers and pasture managers have to rely in the benefits of subsampling techniques. When subsampling, the herbage cutting method (HCM) has been accepted as the most reliable, provided an adequate number of samples are taken. There are, however, some indirect methods that may be easier, cheaper, and faster than clipping, but more information is needed concerning their value under different conditions. Therefore, the objective of this trial was to compare the HCM with three indirect methods for estimating dry matter mass: the pasture capacitance meter (PCM), the pasture plate meter (PPM) and the visual estimation (VE).The trial was conducted on six paddocks of a tall fescue pasture during the growing seasons of 2002 and 2003. Calibration equations were developed for each of the indirect methods on every sampling date using the simple linear regression technique. Data sets for comparisons were analyzed using the mixed procedure of SAS with a model that included paddock as block, sampling method as treatment, sampling dates as repeated measures and the interaction of method by sampling date. The coefficients of determination (r^2) for the pooled calibration equations were 0.686, 0.751, and 0.862 for the PCM, PPM, and VE, respectively. Thus, those values have been considered good enough to estimate forage mass and make pasture management decisions at farm level. There were differences ($P < 0.01$) due to sampling method, date, and their interaction, and individual contrasts show that DM mass obtained by the HCM was overestimated ($P \leq 0.01$) by all three indirect methods. However, the accuracy of these subsampling techniques, compared with total harvest of the pastures, remains to be elucidated.

Key Words: Tall Fescue, Forage mass Estimation, Direct Method, Indirect Methods.

Introduction

Grassland biomass refers to the instantaneous measure of the total weight of herbage per unit area of land, preferably measured to ground level (Hodgson, 1979). This definition implies that the only way to know the pasture DM yield would be by cutting, drying, and weighing the total amount of herbage in the pasture without any loss of material; a difficult and impractical task in grazing studies. Nevertheless, due to the importance of this attribute in making decisions at the farm level and explaining results from experimental trials, pasture managers and researchers usually rely on the benefits of the subsampling techniques to estimate DM mass (Wilm et al., 1944; Mannetje, 2000). When subsampling, direct clipping of herbage provides an objective index of pasture yield, which is accurate, sensitive, and reliable, provided sampling size is adequate. Direct cut sampling, on the other hand, is destructive, requires high inputs of time, labor, and equipment, and may influence production and composition of forage as well as grazing behavior. Thus, the researcher may decide to cut an insufficient number of samples, resulting in low accuracy of the method (Frame, 1981; Mannetje, 2000).

Several indirect methods have been evaluated to estimate forage mass. Some of them include visual estimation (Haydock and Shaw, 1975; Waite, 1994; Smith et al., 2001), plant cover (Anderson and Kothmann, 1982), canopy volume (Thorne, et al., 2002), pasture canopy height (Hutchinson, 1991; Murphy et al., 1995), the use of the pasture plate meter (Bransby et al., 1977; Harmoney et al., 1997; Correll et al., 2003), the single-probe pasture capacitance meter (Vickery et al., 1980; Michell and Large, 1983; Sanderson et al., 2001), visual obstruction (Laca et al., 1989; Vermeire et al, 2002), and more recently, the imaging spectroscopy (Schut and Ketelaars, 2003). Most of these methods have been compared with the HCM in predicting DM yield by the means of regression analysis, but there is a lack of information about the precision of the methods based on actual validation trials. Therefore, the main objective for this trial was to compare the forage mass estimates of the herbage cutting method (HCM) with the estimates of three indirect methods, the pasture capacitance meter (PCM), the pasture plate meter (PPM), and the visual estimation (VE).

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Materials and Methods

This trial was conducted at the Shenandoah Valley Agricultural Research and Extension Center (SVAREC), Steeles Tavern, VA. The SVAREC is located at 37º 56´ North latitude and 79º 13´ West longitude. The main soil type is a Frederick silt loam, mixed mesic, typic paleudult. Pastures typically were predominantly tall fescue (*Festuca arundinacea,* Schreb) but, other species, mainly Kentucky blue grass (*Poa pratensis,* L), orchardgrass (*Dactylis glomerata,* L), quack grass (*Elytrigia repens*), and some broad leaf weeds were also present in different proportions. These pastures were fertilized at a rate of 157-22-45 kg/ha of N, P, and K in 2002, and 157-00-00 in 2003, respectively. Nitrogen was applied twice each year; 67 kg/ha in the middle of April and 90 kg/ha in the middle of August. Phosphorus and K were applied only in April 2002. The forage of these pastures was stockpiled from the second application of N until the end of the growing season. The paddocks included in the study were selected from a large scale cow-calf grazing project grazed in a three paddock method. The criteria to choose the paddocks were the visual appraisals of botanical composition and canopy structure. In order to test the hypothesis of no differences among the sampling methods in forage mass estimations, the four methods were applied to six paddocks on eight sampling dates during the growing seasons of 2002 and 2003 (Table 4).

Table 4. Sampling dates throughout the experimental period

Year	
2002	2003
June 13 and 14	June 5 and 6
August 8 and 9	July 17 and 18
September 5 and 6	September 25 and 26
October 17 and 18	November 10 and 14

Forage mass estimation methods

Direct method. For the HCM, the observational units were strips of 1.5 m^2 (0.5 m by 3.0 m). In each sampling date, five strips per hectare were cut at a height of 2.5 cm above ground level with a lawn mower equipped with a collection bag. The location of the samples was done by a zigzag systematic procedure with different number of observations, depending upon the area of the paddock (Table 5). The sampling method used resulted in a similar number of samples per hectare in all paddocks. The total amount of herbage harvested from each strip was immediately placed in a cloth bag of known dry weight. The sample bags were weighed before and after drying in a forced-draft oven. The oven temperature was maintained at 65 °C to 70 °C and the samples remained in the oven for at least 48 h. Sample DM content was calculated as: DM (%) = (dry weight/fresh weight) X 100, and the dry weight per strip as: DM = (total fresh weight X DM percentage) / 100. The dry weight from the strips was expressed in kg/ha and the estimated DM yield of the paddocks was the mean of these numbers.

Table 5. Paddock identification, area, and sample size

Paddock ID	Area, ha	Strips per paddock	Observations per paddock
K1A	2.459	12	60
K2A	1.912	9	45
H1A	2.459	12	60
H2A	1.912	9	45
B1A	2.459	12	60
B2A	1.912	9	45

Indirect methods. The paddocks were sampled, independently, for each of the three indirect methods. The order of sampling was the PCM, VE and PPM. For the PCM, a commercial pasture probe, GrassMaster II (Tru-Test Ltd., Auckland, NZ.) was used. The GrassMaster II is an easy-to-use pasture probe (weighs about 1140 g), equipped with an electronic processor and indicator that shows the DM mass estimation of up to 200 paddocks, each paddock containing up to 250 readings. Those readings are based on the relationship of capacitance and DM yield of a mixed pasture of rye grass with white clover under New Zealand conditions. Therefore, a calibration equation has to be developed any time the GrassMaster, or any other pasture capacitance probe, is used under different conditions. For the purpose of this experiment, the paddock was walked in a zigzag pattern and a reading with the GrassMaster II was taken and recorded every 15 steps ending up with a total of 45 to 60 observations, depending upon the size of the paddock (Table 5). Each of these readings was used to solve the calibration equation that was developed at the end of the sampling date as described below.

For the PPM, a hand-made manual dropping plate was used. This device consists of a squared acrylic plastic of 0.25 m^2 (0.5 m by 0.5 m) weighing 880 g and having a round hole in the middle. The hole allows the plate to slide freely throughout a PVC pipe of 125 cm in length. This pipe acts as a sleeve for another internal pipe of the same material, but 175 cm in length. The section of this internal pipe that overpasses the external pipe is graduated at a 1 cm scale starting with zero at the level at which the external pipe ends. Both of the pipes have stoppers on one of the edges, the one for the external pipe stops the dropping plate when the operator is carrying the plate and the other keeps the position of the internal pipe at the level of the external pipe. When a measurement is taken, the plate is dropped from about 50 cm above the pasture canopy height, the plate is held by the pasture at a certain height and the internal pipe slides until it touches the ground level. Then the height of the plate can be measured in the scale at the top of the internal pipe. The number of samples taken per paddock varied from 45 to 60 (Table 5).

The VE followed the procedure described for the comparative yield method of Haydock and Shaw (1975) with a five point scale with few modifications. Briefly, the paddock was first walked, when sampling with the PCM, to recognize variation in herbage mass, then visual appraisals of DM yield were made in kg/ha every 15 steps from quadrats of 0.25 m^2 when walking the paddock in a zigzag pattern. The number of observations varied according to the size of the paddock (Table 5).

Once the paddock was sampled with the HCM and the three indirect methods, five quadrats of the same dimensions (0.5 m by 0.5 m) were chosen to build the calibration equation. The condition was that those five quadrats had to cover the range of herbage mass present in the whole paddock, so that most samples were within this range. For each of the quadrats one VE (kg/ha), the average of five PCM readings (kg/ha), and one PPM height (cm), were taken by the same person from the least to the most destructive method. After this, the herbage within the quadrat was cut with hand-held, battery-operated clippers at 2.5 cm above the ground level and the material was placed in a cloth bag and dried as outlined for the HCM.

The calibration equations for each indirect method were developed from the same five standard quadrats chosen per paddock, making up a simple linear regression with a total of 30 points for each of the indirect methods, per sampling date. The regressors or independent variables were the DM mass (kg/ha) estimated by the observer in the VE method, the DM mass reading on the PCM, and the height (cm) recorded for the PPM. The response variable for each of the method was the actual forage mass of the quadrats (kg/ha). In order to have an estimation of the paddock's DM mass per method, the linear regression equation obtained for each method was solved for each of the observations taken when sampling the whole paddock with the respective indirect method. The five points from the quadrats used to build the calibration equation were added to the number of observations per paddock. The arithmetic mean of these 50 to 65 observations was the forage mass estimate for the respective paddock and sampling method.

Statistical analyses

In this trial, accuracy was tested with regression analysis whereas precision was evaluated applying ANOVA to the outputs in DM mass from the four sampling methods. All data sets were analyzed using the version 8.2 of the SAS (2001) according to Littell et al. (2002). The calibration equations for the indirect methods for each of the sampling dates and the pooled regression equations across sampling dates within years were calculated using the regression procedure with the simple linear regression model. In order to compare the slopes of the regression lines for sampling method and dates, the working hypothesis of equality of the regression coefficients was tested using the GLM procedure. For the whole period of the study, ANOVA was carried out using the mixed procedure in order to test the null hypothesis of no difference among the methods in DM yield estimations. Paddock was considered the experimental unit to which the treatments (methods) were applied. The model included only the fixed effects of paddock as block, sampling method as treatment, with sampling dates as repeated measures, and the interaction of method by sampling date. Whenever the ANOVA detected any significant difference ($P \leq 0.05$), linear contrasts were used to compare the mean DM mass estimated with the direct HCM method with that obtained with each of the three indirect methods. Also, year 2002 was contrasted with year 2003. The SAS codes are shown in Appendix I.

Results and Discussion

Accuracy of the indirect methods

All the regression equations of actual forage mass on records from the three indirect methods (Appendixes II, III, and IV) were significant ($P < 0.001$) as it was suggested due to the standardization procedure of Haydock and Shaw (1975). With the exception of the sampling date of Aug. 8, 2002, all the r^2 are acceptable, ranging from 0.879 to 0.942, from 0.816 to 0.931, and from 0.726 to 0.898 for the VE, the PPM, and the PCM, respectively. These r^2 values are better than those found by Bransby et al. (1977), Murphy et al. (1995), and Rayburn and Rayburn (1998), and are comparable with

those found by Stockdale and Kelly (1984), Griggs and Stringer (1988), and Harmoney et al. (1997). When the regression coefficients were tested for equality, there was no difference in coefficients among sampling methods (P = 0.815) or dates (P = 0.379) which, gave rise to the pooled regression analysis. The pooled calibration equations are shown in Table 6. Regressions by year are presented because the weather conditions at the experimental site were very different. Year 2002 was hot, and dry with a total annual precipitation of 883 mm whereas yr 2003 had a more mild summer season with a total of 1719 mm of precipitation.

Table 6. Pooled calibration equations of actual forage mass on records from the pasture capacitance meter, the pasture plate meter, and the visual estimation

Sampling year	Regression***				Actual DM mass, kg/ha[c]	RSD, kg/ha[d]
	β_0	β_1	$SE(\beta_1)$[a]	r^2 adj[b]		
Pasture capacitance meter, kg/ha						
2002	-511.434	1.483	0.125	0.540	2256	1137
2003	66.925	1.605	0.960	0.700	4219	1509
Pasture plate meter height, cm						
2002	-466.302	248.779	14.155	0.721	2256	885
2003	-691.871	305.961	17.395	0.721	4219	1456
Visual estimation, kg/ha						
2002	127.415	1.714	0.075	0.814	2256	723
2003	-5.506	1.816	0.071	0.844	4219	1087

*** All regression ($Y_i = \beta_0 + \beta_1 X + E_i$) were significant (P < 0.001).

[a] Standard error of the regression coefficient (β_1).

[b] Adjusted coefficient of determination.

[c] Dry matter yield estimated from the cut quadrats.

[d] Residual standard deviation, n = 120.

The r^2 value for the PCM was better in 2003 than in 2002. The malfunction of the pasture probe in yr 2002, and especially in summer 2002 ($r^2 = 0.491$, Appendix II), was likely due to the lack of contact between the plates of the capacitance system which, in turn was probably affected by the lack of soil moisture and forage in the sampling sites. Also, the PPM and the VE methods had low r^2 values (0.600 and 0.795, respectively) during this sampling date of summer 2002. These results are in agreement with those found by Vartha and Matches (1977), Michell and Large (1983), and Douglas and Crawford (1994) who warn about the use of the PPM when the forage mass is too low or too high with stemmy or trampled plants.

In this trial, the r^2 and residual standard deviation values from the pooled regressions are similar to those reported in the literature (Michalk and Herbert, 1977; Griggs and Stringer, 1988; Gabriels and Van Den Berg, 1993). In the present study, those values show that the accuracy of the indirect methods was best for the VE, followed by the PPM, with the worst results for the PCM. In contrast, Stockdale and Kelly (1984) and Murphy et al. (1995) found better r^2 and coefficients of variation for the PCM than for the PPM. Overall, the conclusions of these reports are that the accuracy of the double sampling techniques is good enough to estimate forage mass and take daily decisions in pasture management at the farm level.

The calibration equations of this trial are better for the VE method, with the pooled regression model able to explain up to 86% of the total variation and a residual standard deviation of about 28% of the mean forage mass. The problem with this method is that it is highly subjective and there may be individual differences in visual appraisal of any trait in the sward, as it was found by Aiken and Bransby (1992). Results for the PPM show r^2 values between those of the VE and the PCM with their residual standard deviations among the best of this trial. Compared with VE, the PPM is a more objective method to estimate forage DM mass. The PPM is also fast, inexpensive, and easy to use as discussed by Michell (1982), Griggs and Stringer (1988), and Douglas and Crawford

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(1994). It shows potential, not only on estimations of grassland biomass but also in studies of spatial and temporal dynamics of the sward (Correll et al., 2003).

The relatively large residual standard deviation values found in this trial may indicate the large variation in forage mass found in these types of pastures, but also may reflect the variation due to the standardization of the sampling procedure. The five quadrats collected within each of the six plots were chosen with the condition that they would represent the whole variation within the paddock. This method (Haydock and Shaw, 1975) can reduce the number of paired samples that have to be taken in order to calibrate the methods, but it may also give large residual standard errors. The usefulness of the pooled regression equations is that they have been drawn from a large sample size taken at different points in time and therefore include variations in weather conditions, pasture management, herbage mass, and plant maturity. If someone would like to use one of these methods to estimate the forage DM mass of a mature, grazed, tall fescue pasture without making calibrations, the recommendation would be to use the regression of yr 2002 if the weather conditions have been hot and dry. If the climate has been humid and cool, it would be better to use the regression developed for yr 2003. When no care is taken about climate, the total pooled regression would be more advisable.

Precision among methods

The ANOVA showed differences due to sampling method ($P < 0.001$), sampling date ($P < 0.001$), and their interaction ($P = 0.007$). The difference in dates was expected because the paddocks were sampled during the grazing seasons of yr 2002 and 2003 and most likely the forage mass changed with time. The dynamics in pasture DM during the period of the study is shown in Figure 1. Two things deserve attention in the figure. First, yr 2003 was better then 2002 with regard to forage production. Most likely this was a consequence of the better climatic conditions as explained above. In 2002, the average DM yield was only 1558 kg/ha, whereas in 2003 it was up to 3559 kg/ha. Secondly, DM yield was greater at the end of the growing season because the forage was stockpiled.

This is a common practice with tall fescue pastures which, at the same time provided us with the opportunity of testing the methods under a wide range of forage mass.
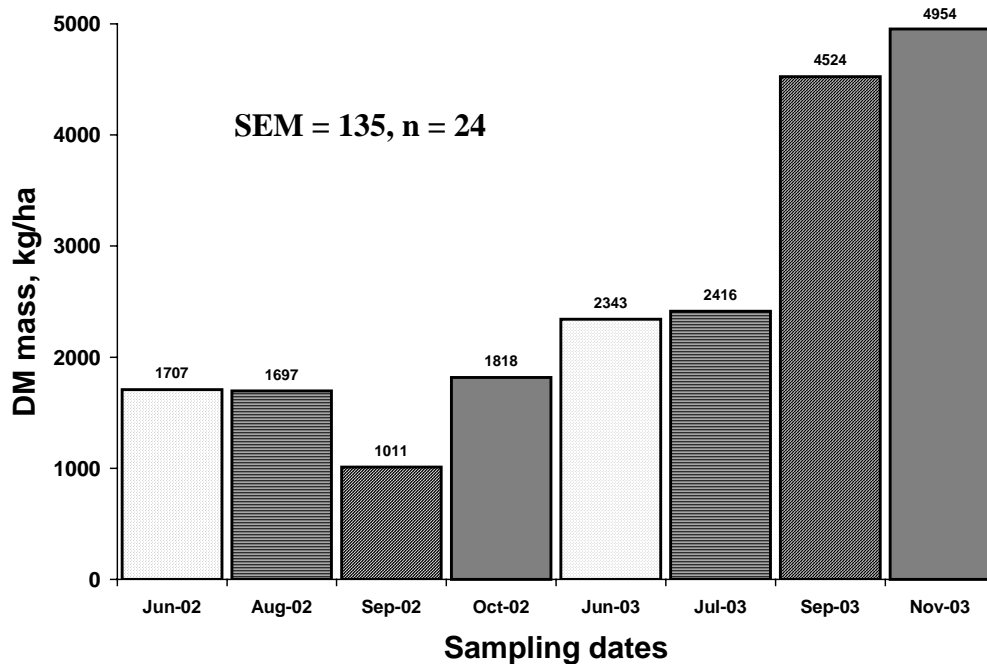
Figure 1. Estimated forage mass during the experimental period, average of all methods.

Sampling methods were expected to be similar in estimating pasture DM yield, which was not the case. Moreover, when using the direct HCM as the method of reference, there were differences ($P < 0.001$) between this method and each of the three double sampling techniques (Table 7). The contrast for years also showed difference ($P < 0.01$) in estimated forage mass between 2002 and 2003. When the ANOVA was done by year, the differences in main effects persisted ($P \leq 0.03$) but their interactions with sampling dates became non-significant ($P \geq 0.072$). These results, along with the individual contrasts for the HCM with each of the three indirect methods, are shown in Appendix V.

Unlike the results from Michalk and Herbert (1977) and Sanderson et al. (2001), this research indicates that the indirect methods overestimated the DM yield obtained

with the HCM. However, no difference was found among the indirect methods (P ≥ 0.05). In this trial, we did not have the actual measure of pasture yield, but only got estimations through the methods in study. It would be desirable to compare these subsampling procedures with the total harvest of the pasture.

Table 7. Pasture forage mass estimates with four methods

Sampling method	DM yield, kg/ha	Contrast P value[a]
Herbage cutting method	1953	-------
Pasture capacitance meter	2730	< 0.001
Pasture plate meter	2862	< 0.001
Visual estimation	2689	< 0.001
SEM[b]	124	

[a] Observed significance level for the linear contrast of the
   herbage cutting method vs. the respective indirect method.
[b] Standard error of the mean, n = 48.

Generally, in these kind of studies most of the researchers accept that the HCM provides the most reliable biomass estimation with the condition that the number of cut samples is adequate. Therefore, if five 1.5 m^2 strips per hectare were an adequate sample size for the conditions in this trial then, the indirect methods would not be acceptable. If the 26 observations (on 0.25 m^2 quadrats) per hectare taken with the indirect methods were able to provide a more accurate estimation of the forage mass then the HCM would be questioned. Michalk and Herbert (1977), working with alfalfa pastures, and Murphy et al. (1995), working with ryegrass, tall fescue, and ryegrass/clover pastures favor the use of the non-destructive techniques but others (Gabriels and Van Den Berg, 1993; Sanderson et al., 2001) stated that … "at present it is impossible to make an accurate prediction of the DM yield of a certain site based on the non-destructive measurements".

Meanwhile we can only conclude that the direct subsampling HCM in this experiment gave estimations consistently lower than the three indirect double sampling techniques of PCM, PPM, and VE. The reliability of these methods to estimate the actual pasture DM yield remains to be elucidated, preferably by harvesting the whole plots.

Implications

Pasture dry matter yield is a basic measurement in forage evaluation and grazing trials. However, in most of the grazing conditions, forage mass can only be estimated using one of the several subsampling techniques available. This study emphasizes the need of being careful when selecting the method to estimate pasture dry matter mass because they may render different estimates of the same forage crop. Sampling intensity, size, and shape of the quadrat play an important role when using the direct and destructive cutting method. Accuracy of the calibration equation and sample size are key elements when using a double sampling technique.

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CHAPTER IV

Manuscript 2. Recovery rates of chromic oxide and alkanes from controlled release capsules and concurrent estimations of intake and digestibility in beef steers

ABSTRACT. A digestion trial was conducted at the Virginia Tech Smithfield Barn. Six Angus crossbred steers (avg. BW 328 ± 31 kg) were used to determine the recovery rate (RR) of chromic oxide (Cr_2O_3) and alkanes, and to assess the accuracy of the indicators to evaluate DM fecal output (DMFO), intake (DMI), and digestibility (DMD). Steers were allotted to individual pens and were fed tall fescue hay at a daily rate of 1.5% BW. Daily allowance of hay was offered twice per day in two equal portions. Seven days before the beginning of the collection period, the steers were dosed with two intraruminal controlled release capsules (CRC), one containing Cr_2O_3, and the other containing a mixture of alkanes C_{32} and C_{36}. The release rate stated by the manufacturer was 1.5 g/d of Cr_2O_3 and 0.4 g/d of alkanes. During the collection period (7 d), fecal collection bags were changed twice per day at 0700, and at 1600 and a 5% sample was collected from each bag after thoroughly mixing the feces. Rectal grab samples were also taken from each animal in the morning and in the evening when changing the bags. All fecal samples were stored at -18 ºC pending analysis. Hay samples, representative of each meal, were also collected and store at room temperature. Laboratory analyses included DM, ash, CP, NDF, ADF, IVDMD, Cr_2O_3, and alkanes. Data were analyzed with repeated measures, using the mixed procedure of SAS. Actual average DMI, DMFO, and DMD were 4.735 kg/d, 1.847 kg/d, and 61%, respectively. Alkane C31 was found in higher concentration than other alkanes in hay and feces. No differences were found among steers for the recovery rates of either alkanes (P ≥ 0.106) or Cr_2O_3 (P = 0.341) during the 7 d of the collection period. Likewise, actual DMFO, DMI, and DMD were not different (P ≥ 0.161) from the estimated values when using alkanes C_{32} and C_{36} or Cr_2O_3 as external markers. However, these estimated values had to be corrected by the RR of the respective markers. With the double alkane technique only the fecal ratios C_{32}/C_{31} and C_{36}/C_{31} gave reliable estimates of DMI. The use of the pooled fecal samples also gave reliable estimates of the average daily DMFO, and DMI during the collection period.

Key Words: Digestion Trial, Beef Steers, Tall Fescue, Alkanes, Chromic Oxide.

Introduction

Dry matter intake and digestibility represent useful estimates of forage quality when animal production cannot be measured (Sollenberger and Cherney, 1995). When the nutritive value, or quality of forage, is measured directly from the animals, it provides a good estimate of the potential that a given feed has to sustain the animal functions of maintenance, growth, lactation, and others. The nutritive value is considered to be a result of intake, digestibility and animal efficiency to use the available nutrients. Of these factors, intake and digestibility account for most of the variation in animal performance. Therefore, they are the most common indicators of forage quality indices, such as "the relative feed value" and "the relative forage quality" described by Moore and Undersander (2002). In a conventional digestion trial, both feed intake and total fecal output can be measured directly. Apparent digestion coefficients are easy to calculate as the difference between DMI and fecal DM excreted divided by the DMI (Merchen, 1988). Unfortunately, total fecal collection is not always possible in grazing trials. Thus, neither the animal intake nor the digestibility of the forage eaten can be measured. Under these conditions, the use of some inert reference substances, known as markers, probably represents the most widely accepted method to estimate both animal voluntary feed intake and forage digestibility (Van Soest, 1994).

Kotb and Luckey (1972), Ellis et al. (1980), and Owens and Hanson (1992) have made a broad classification of these non-absorbable, nutritional markers as internal or external depending upon their natural appearance. Merchen (1988), and Marais (2000) made a general description of the physical and chemical characteristics and properties of the most common markers. These authors indicated that probably none of the actual markers fulfill all the requirements, and that method of administration plays an important role in the accuracy of the estimates of fecal output and thereby, DM intake and digestibility calculations.

Gelatin capsules, paper tissue, fiber fractions and other substances have been used as carriers for markers in digestion trials with cattle. Recently, the use of intraruminal controlled release capsules has been a popular means to dose animals with markers such as chromic oxide and long chain alkanes to estimate intake and digestibility of grazing animals (Milne, 2001). Information about the accuracy of these methods is still needed. Therefore, the objectives of this experiment were to:

a) Determine the recovery rate of chromic oxide and long chain alkanes from controlled release capsules administered to steers fed tall fescue.

b) Assess the value of the markers for the concurrent estimations of dry matter fecal output, intake, and digestibility.

Material and Methods

This trial was conducted from March 07 through July 12, 2003 at the Smithfield barn, Virginia Tech., Blacksburg, VA. The total period of time was divided in the training and the experimental phases. At the beginning of the trial, six Angus crossbred steers (avg BW 328 kg ± 31 kg) were randomly allotted to individual pens of 3.35 m in length and 2.30 m in width (7.7 m^2). The steers were fed a standard high-roughage diet containing (as-fed basis) grass hay (50.4%), ground corn (40.5%), wet sugar cane molasses (5.0%), soybean meal (3.5%) and trace mineralized salt (0.6%). The level of feeding was 1.5% BW, as fed basis. One half of the daily individual diet was given at 0800 h and the other half at 1700 h. Steers had access, at all times, to fresh water and trace mineral salt bricks containing (as-fed basis) salt (98.5%), zinc (0.35%), iron (0.20%), manganese (0.20%), copper (0.03%), iodine (0.007%), and cobalt (0.005%) (Champion Choice, Cargill Inc., Minneapolis, MN).

During the training phase, the diet of the steers was changed gradually from the high roughage to tall fescue (*Festuca arundinacea*, Schreb) hay. The feeding level of 1.5% BW (as fed basis) was held constant, as well as the way the diet was provided twice per day. The tall fescue hay was ground prior to its administration in order to avoid selection and wastage from the trough. The stepwise substitution was done at 20% daily

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from April 07 through April 12, 2003. Once the steers were adapted to the pens and the diets, they were halter broken. Once the animals were tamed enough they were trained with the harnesses and fecal collection bags (Hanstings Canvas Manufacturing Co., NB, USA), working outside the pens for about 30 min per animal every other day. When the experimental phase began, the steers were already adapted to the diet and management.

The experimental phase began on June 18, 2003 (d 1). On the morning of that day, the steers were weighed (at 0800) and dosed (at 0930) with two intraruminal controlled release capsules (CRC) for large cattle (300 to 700 kg) in the manner stipulated by the manufacturer (CAPTEC Lty. Distributed by Nufarm, Auckland, NZ). One of the CRC contained chromium sesquioxide (Cr_2O_3) and the other, the alkanes n-dotriacontane (C_{32}) and n-hexatriacontane (C_{36}). The release rates stated by the manufacturer for these capsules were 1.5 g/d for the Cr_2O_3 and 0.4 g/d for the n-alkanes capsules. On d 6 of this phase, the steers were fitted with the fecal collection bags. On the morning of d 8 (June 25, 2003), fecal collection was started using the procedure depicted in appendix VI. Briefly, the fecal collection bags were changed twice per day, at 0700 and at 1600. The content of each bag was weighed, thoroughly mixed, and 5% samples were taken from different spots and sealed in double plastic bags. The fecal collection period was of 7 d and all the samples were stored in a freezer at -18 ºC.

In addition, rectal grab samples were collected from each of the steers on the mornings (at 0700) and evenings (at 1600) during the 7 d of the collection period. These samples were taken during the change of the feces collection bags, placed in sealed plastic bags and stored under frozen conditions (-18 ºC) pending future analysis.

Samples of hay that represented the individual morning and evening meal were also taken during 7 d, from 2 d before the beginning until 2 d before the ending of the fecal collection period. These hay samples were placed in double plastic bags and stored at room temperature. One of the steers (ID # 121) refused a little of the hay on d 4 and d 5 of the collection period. These refusals (orts) were weighed and sealed in plastic bags in the freezer for chemical analyses.

Laboratory procedures

All samples were handled and analyzed, in duplicate. The morning and evening hay samples of each steer were mixed by day and the resulting composite samples (1 per steer, per d) were ground in a Wiley mill (Thomas Wiley, Laboratory Mill Model 4, Arthur H. Thomas Co. Philadelphia, PA) to pass through a 1 mm screen. These samples were analyzed for DM, ash, NDF, ADF, and IVDMD. In order to determine CP, Cr_2O_3 and alkane concentrations, a subsample of this material was ground to pass through a 0.5 mm sieve. The orts from the steer 121 were handled as the hay samples.

Fecal samples collected from the bags were thawed at room temperature for about 24 h, then were mixed and two subsamples were taken. The first one, containing about 100 g of fresh material, was dried in a forced-air oven for 24 h, ground in a Wiley mill to pass through a 1-mm screen and analyzed for DM, ash, NDF, ADF, and IVDMD. The second subsample, containing about 20 g of fresh material, was placed in a beaker, covered with cheese cloth and freeze dried in a FreeZone 12 Liter lyophilizer (LABCONCO, Co. Kansas City, MO, USA). This dry subsample was ground in a cyclone mill to pass through a 0.5-mm screen and it was analyzed for DM, ash, CP, n-alkanes, and Cr_2O_3 content.

The fecal grab samples were mixed by steer across the collection period (7 d), ending up with one AM and one PM grab sample per steer. These samples were handled, freeze dried, and ground as the second fecal subsample from the bags. They were analyzed for alkanes and Cr_2O_3 content.

Dry matter and ash content was determined in ground samples from hay, feces, and grabs following the AOAC (2000) procedure. Briefly, 0.5 g of the samples were placed in a crucible and dried for 24 h in a forced-air oven at 100 ºC. Ash determinations were made in sequence leaving the samples in a muffle furnace for 3 h at 500 ºC. Crude protein was obtained by analyzing N content of the samples in a ParkingElmer 2410

Nitrogen Analyzer (PerkinElmer Instruments LLC. Shelton, CT, USA), according to the AOAC (2000) procedure. The NDF and ADF determinations were made according to AOAC (2000) procedure with the ANKOM 200/220 Fiber Analyzer following the manufacturer's procedure recommended in the operator's manual (ANKOM Technology, 1997a). The IVDMD was determined using the DAISY II$^{200/220}$ Rumen Incubator (ANKOM Technology, 1997b).

Chromic oxide concentrations in hay, feces and orts were determined according to AOAC (2000) with modifications. Wet ashing was done using a 2:1 (vol:vol) solution of HNO_3 and $HClO_4$ on 0.5 g of ground sample as described by Muchovej et al. (1986). Final Cr_2O_3 concentrations were determined by atomic absorption spectrophotometry in a PerkinElmer Analyst 800 (PerkinElmer Instruments, Uberlingen, Germany). The chromium standard curve was determined using a solution prepared at 0.0, 1.0, 2.0, 3.0, and 4.0 ppm using a chromium reference solution (Fisher Scientific, NJ, USA).

Alkane analyses were conducted on the freeze-dried and ground subsamples following the procedure outlined by Mayes et al. (1986) with some modifications. Briefly, samples of feces, hay, and orts weighing 0.1, 0.3, and 0.3 g, respectively, were placed along with 0.0010 g of the internal standard (alkane C_{34}) in a 20 mL pyrex tube fitted with a screw cap. The samples underwent direct saponification with 7 mL of a 10% ethanolic KOH solution at 90 ºC for 3 h in a water bath with mixing of the contents every 30 min. After cooling, 7 mL of deionized water and 7 mL of heptane were added and the tube contents were mixed. The organic extract was removed, applied to a filtration column, and eluted with about 10 mL of heptane into a 20 mL scintillation vial. Filtration columns were made of 5 mL disposable pipettes containing silica gel with glass wool stoppers. The eluent was then dehydrated in a N evaporator unit (Organomation Associates, Inc. Berlin, MA. USA). When ready for analysis, the residue was re-dissolved with 1 mL of heptane before injection of 0.5 μL into the capillary column of a gas chromatograph (Agilent Technologies 6890. Santa Clara, CA) equipped with flame ionization detector, integrator, and a 7683 series autosampler. The column specifications were 30 m long, 0.52 mm i.d., and 1.5 μm of fused silica film thickness (Supelco Inc.

Bellefonte, PA. USA). Oven temperature was programmed to hold at 240 ºC for 4 min, increased to 288 ºC at 3 ºC/min, and then increased to 298 ºC at 2 ºC/min. Helium was used as the carrier gas with a flow rate of 9.0 to 9.25 mL/min. The identity of alkanes was determined from their retention times relative to known standards, whereas peak areas were converted to amounts of alkanes by reference to the internal standard.

Measured and estimated variables

All the calculations were made on DM basis with the following expressions:

1. Actual DM intake = DMI (g/d) = Amount of hay (offered - refused).

2. Actual DM fecal output = DMFO (g/d) = (Morning + Evening) Collection of feces
   Note: 150 g were added per bag to compensate for the grab sample collected.

3. Apparent DM digestibility = DMD (%) = [(DMI - DMFO) / DMI] X 100.

4. Nutrient composition of hay, feces, and orts as described above.

5. Concentrations of internal and external markers in hay, feces, and orts as above.

6. Markers recovery rates (RR) = Marker in feces (g/d) / daily dose of marker (g/d).

7. Estimated DM fecal output (DMFOE) with the use of external markers.
   DMFOE (g/d) = [Daily dose of marker (g/d) / Marker concentration in feces
   (g/g DM)] X RR.

8. Estimated DMI (DMIE) with the use of external markers and the IVDMD
   DMIE (g/d) = DMFOE (g/d) / (1 - IVDMD).

9. Estimated DMI (DMIE) with the alkanes technique

DMIE = Dj / [(Fj/Fi) X Hi-Hj]

Where:

Dj = dosed even-chain alkanes.

DMIE = Dry matter intake estimation (g/d).

Fj and Fi = fecal concentration (g/g DM) of even (j) and odd (i) chain alkane.

Hj and Hi = herbage concentration (g/g DM) of even (j) and odd (i) chain alkane.

10. Estimated DMD with the natural odd chained alkanes as internal markers

DMDE = [1 - (RRi X (Hi / Fi))] X 100

Where:

DMDE = Dry matter digestibility estimated (%).

RRi = Recovery rate of the odd chained (i) alkane used in the formula.

Hi = Concentration of the i-th alkane in the herbage.

Fi = Concentration of the i-th alkane in the feces.

In addition, the grab samples were used to determine Cr_2O_3 concentrations, recovery rates, and DMI throughout the fecal collection period. Alkanes were not used for these calculations because of a problem with the alkane program in the GC when running these samples.

Statistical analyses

This trial corresponded to a randomized complete block design in which, the six steers were used as the experimental units, treatment was the method used to estimate the response variables (DMFOE, DMIE, DMDE) and days of the collection period (7 d) were considered repeated measures on time. Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA, 2001) according to Littell et al. (2002). Linear contrasts were used to compare the actual vs. the estimated variables. Effects were considered significant when the P value was lower than 0.05 or with a trend when the P value was between 0.05 and 0.10. The data from the grab samples were also analyzed with the mixed procedure of SAS using the AM and PM samples as repeated measures

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because the samples were pooled across the seven days of the collection period. The SAS codes for these analyses are shown in appendix VII.

Results and Discussion

During the 7 d of the collection period, the average DMI of the steers was 4.735 kg/d (1.41% BW) of hay with 94% DM. Average DMFO was 1.847 kg/d. Thus, apparent in vivo DMD of the hay was 61%. Table 8 shows the nutrient content, on DM basis, of the tall fescue hay used in this trial. Apparently, the hay was of regular quality and its composition did not change ($P \geq 0.109$) during the trial.

Table 8. Average nutrient content (DM basis) of the hay.

Item	Percent	SEM [a]
Component		
Ash	8.19	0.066
CP [b]	11.12	0.089
NDF [c]	54.98	0.722
ADF [d]	29.16	0.435
IVDMD [e]	64.16	1.235

[a] Standard error of the mean (n = 42).

[b] Crude protein.

[c] Neutral detergent fiber.

[c] Acid detergent fiber.

[e] In vitro dry matter degradability.

Content of alkanes in hay and feces

Table 9 shows the alkanes profile in hay and feces during the collection period. The hay content of alkanes other than C_{29}, C_{31}, and C_{33} was very low (less than 10 mg/kg) and several times they did not appear in the chromatogram. These results are in agreement with those from Piasentier et al. (1995) who found that in tall fescue, the most common n-alkanes were C_{29}, C_{31}, and C_{33} with a predominance of C_{31} (about 62% of the total). Alkane concentration in temperate forage plants seem to follow a curvilinear relationship, which depends on the carbon chain length of the odd-chain alkane, with alkane C_{31} being the most abundant (Malossini et al., 1990; Boadi et al., 2002; Bugalho et al., 2004). Moshtaghi Nia and Wittenberg (2002) found that the odd-chain alkanes in bromegrass and alfalfa samples represented an average of 95% and 93% of the total C_{25} to C_{36} alkanes and that C_{31} comprised 43% and 66% of the total odd chain alkanes in bromegrass and alfalfa, respectively.

Table 9. Average alkane content (DM basis) on tall fescue hay and feces

Item	Alkanes				
	C_{29}	C_{31}	C_{32}	C_{33}	C_{36}
Hay	----------------------------mg/kg----------------------------				
Avg.	50	143	---	39	---
SEM [a]	5.36	12.51	---	2.72	---
Feces					
Avg.	111	373	171	125	214
SEM [a]	9.00	27.88	11.55	7.29	9.64

[a] Standard error of the mean (n = 42)

If reliable estimates of DMI are to be calculated, it has been suggested that the concentration of the natural odd chain alkanes should exceed 50 mg/kg (Casson et al., 1984; Laredo et al., 1991). This partially explains why C_{31} and C_{33} are the natural alkanes most widely used to estimate both DMI and DMD (Mayes et al, 1986; Dove and Mayes, 1991, 1996). Odd-chain Alkane concentrations were higher in fecal than in herbage samples (Table 9) as it was expected due to the DMD of the diet. However, the presence of C_{32} and C_{36} in feces is due to the release of the alkanes from the intraruminal CRC since the hay was practically devoid of C_{32} and C_{36} alkanes.

Marker's recovery rates.

There were no differences in the RR of either alkanes ($P \geq 0.106$) or Cr_2O_3 ($P = 0.341$) during the 7 d of the collection period (Figure 2).
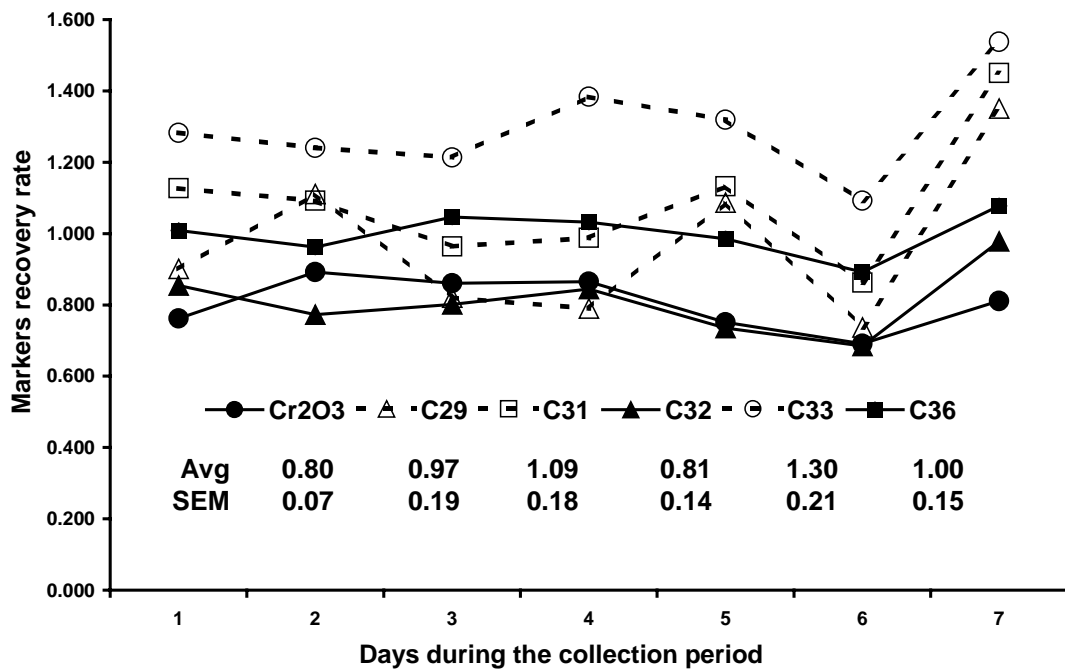

Figure 2. Average chromic oxide (Cr_2O_3) and natural (C_{29}, C_{31}, and C_{33}) and dosed alkanes (C_{32}, and C_{36}) recovery rates from seven beef steers fed tall fescue hay in a digestion trial.

In calculating DMI with the alkane method, it is a prerequisite that the pair of alkanes used in the fecal ratio (F_j/F_i) of the formula, have a similar RR (Mayes et el., 1986) which is not always the case. Frequently, the alkane RR follows a curvilinear and asymptotic relationship dependent on the carbon chain length of the alkanes (Mayes and Lamb, 1984; Casson, 1990; Dove et al., 2002). At other times, the RR does not follow any clear relationship with the carbon chain length (Vulich et al., 1991; Moshtaghi Nia and Wittenberg, 2002; Hendricksen et al., 2002). In this trial, it seems that the intraruminal CRC released a constant amount of the external markers. Similar results have been found by other authors when evaluating the use of CRC in cattle (Brandyberry et al., 1991; Momont et al., 1994) and sheep (Parker et al., 1990; Luginbuhl, et al., 1994; Dove et al., 2002). In general, they have observed a lag phase in the marker appearance in feces of about 2 to 3 d, then, a constant increase in marker concentration, and finally, after 5 to 7 d of the CRC administration, they observed a steady state that may last from 20 to 25 d. For this trial, the average RR of alkane C_{36} seems to be perfect (1.00) but that of C_{32} (0.81) and Cr_2O_3 (0.80) are below the averages found in the literature (Le Du and Penning, 1982; Dove and Mayes, 1996). These low RR are more similar to those found by Moshtaghi Nia and Wittenberg (2002) who evaluated the use of CRC to dose Holstein steers with Cr_2O_3 or alkanes in a digestion trial. When the steers ate bromegrass hay without supplements the average recovery rate of the marker was 0.82 and 0.84 for Cr_2O_3 and alkane C_{32}, respectively. In other studies, it has been demonstrated that alkanes of different chain length can disappear from different sections of the gastrointestinal tract of the ruminants (Dove and Mayes, 1991; Ohajuruka and Palmkist, 1991) which might explain the low RR of C_{32} in the present study. Lewis et al. (2003) stated that some of the apparent variation in alkane concentrations reported in the literature could be due to differences between laboratories in analytical methods.

With regard to the low RR of Cr_2O_3, it is known that this marker tends to travel as a suspension in the digesta at a rate independent of that of either the particulate or the liquid phases (Merchen, 1988) and that due to its density it may remain in some parts of the digestive tract (Ehle et al, 1984; Marais, 2000). However, there is a question about the actual amount of the marker that is being released by the CRC (Burns, 1994). It is also

possible that the solubility of the matrix substance containing the markers is affected by changes in the ruminal environment, such as changes in pH, microbial populations, and molar concentration of compounds (VFA, NH_3, CH_3, etc.). Those changes could explain, at least in part, the variations found in markers RR from CRC due to type of diet and class of animal (Pond et al., 1990; Burns, 1994; Moshtaghi Nia and Wittenberg, 2002). Therefore, verification of the marker results by comparisons with total fecal collection and corrections by the actual RR are advisable (Hatfield et al., 1991; Momont et al., 1994) when working with CRC to estimate DMI and DMD.

Dry matter fecal output, intake, and digestibility

When DMFO and DMI were actually measured and estimated with Cr_2O_3, and alkanes C_{32} and C_{36} as external markers, there were no differences due to method (actual and estimated), day of the collection period, or the interaction (method by day, Table 10). Animal excretion and feed intake were estimated reliably by the two types of markers combined with the hay IVDMD.

Estimates with Cr_2O_3 reflect better estimates than the estimates with alkanes, with an overestimation of less than 2% in DMI. These results agree with those of Momont et al. (1994) who also reported an estimation of DMFO and DMI less than 2% different than the actual values when the Cr_2O_3 concentration was determined from subsamples of the total feces collected. In contrast, the results reported by Brandyberry et al. (1991), Hatfield et al. (1991), and Buntix et al. (1992) when evaluating the use of Cr_2O_3 CRC with sheep and cattle consuming different diets, indicate that estimates of DMFO and DMI were not close to the actual values. Nonetheless, although the RR was determined or was calculated in all those trials, none of the authors used it in the calculations, as it was done by Momont et al. (1994) and in this report.

The use of even-chain alkanes to estimate DMFO with digestion coefficient is not common in the literature for estimates of forage intake. However, as it is done with Cr_2O_3 or any other external marker they can be used for that purpose if an accurate RR can be

attained (Mayes et al., 1995). Table 10 shows that no difference was found between actual and estimated DMFO and DMI using either C_{32} or C_{36} from CRC and the hay IVDMD. More commonly, an even-chain alkane is coupled to an odd-chain alkane of similar RR that is usually adjacent in carbon chain length in order to estimate herbage intake. The most common pair of alkanes used in the fecal ratio of the formula to estimate herbage intake is the C_{32} and C_{33}. Perhaps that is because it has been reported (Dove and Mayes, 1991) that the average RR of C_{32} is 0.868 and that for C_{33} is 0.872, close enough to give a good prediction of DMI.

Table 10. Effect of external marker upon dry matter fecal output (DMFO) and intake (DMI) estimates, and comparisons between actual and estimated values.

Method	DMFO,[a] kg/d	DMI,[b] kg/d	Contrast P value for actual versus estimated [c]	
			DMFO [a]	DMI [b]
Actual	1.847	4.735		
Estimated				
Cr_2O_3	1.889	4.812	0.887	0.891
Alkane C_{32}	2.007	5.236	0.315	0.381
Alkane C_{36}	1.925	4.992	0.635	0.650
SEM [d]	0.148	0.395		

[a] Dry matter fecal output corrected by the respective recovery rate.

[b] Dry matter intake corrected by the respective recovery rate.

[c] Observed significance level for the linear contrasts.

[d] Standard error of the mean (n = 42).

Unfortunately, there is a lot of variation in both the alkanes content and RR among the reports found in the literature, with more variation found in trials conducted

with cattle (Mayes et al., 1986; Moshtaghi Nia and Wittenberg, 2002; Hendricksen, 2002) than those conducted with sheep (Mayes et al., 1986; Vulich et al., 1991; Dove et al., 2002).

The results of this trial when different alkane ratios were used to estimate DMI by the double alkane technique are shown in Table 11. The estimated DMI differed (P < 0.001) from the actual value when using the fecal ratios C_{32}/C_{33} and C_{36}/C_{33} which disagrees with the general suggestion of using the pair C_{32}/C_{33} in calculating herbage intake (Dove and Mayes, 1991; Mayes et al., 1995). However, when using the alkane ratios C_{32}/C_{31} and C_{36}/C_{31}, there was no difference (P $\geq$ 0.270) between the actual and estimated DMI. That is likely due to the similarity in RR found between the even chain alkanes C_{32} (0.81) and C_{36} (1.00) and the odd chain alkane C_{31} (1.09).

Table 11. Least square means for actual and estimated dry matter intake (DMI) using different fecal ratios with the double alkane procedure

Method	DMI, kg/d	SEM (n)
Actual	4.735	0.238 (42)
Estimated with the Alkane ratio:		
C_{32}/C_{31}	5.116	0.244 (40)
C_{32}/C_{33} [a]	6.173	0.244 (40)
C_{36}/C_{31}	4.960	0.243 (40)
C_{36}/C_{33} [a]	5.988	0.243 (40)
P value [a]	< 0.001	

[a] Different (P < 0.01) from actual

Berry et al. (2000) conducted a digestion trial to test the use of the alkanes (C_{32}, C_{36}) CRC in estimating herbage intake by Brown Swiss cows. They found that the best

results were attained when the C_{32} / C_{33} alkane ratio was used, due to the small difference (0.02) in RR between them. Contrary to our results, C_{36} had a low RR (0.81) that affected its use in combination with either C_{31} (RR = 0.76) or C_{33} (RR = 0.85). In general, the closer the RR of the alkanes pair the more reliable the DMI estimation; our results indicate an overestimation of less than 5% when using the ratio C_{36}/C_{31}.

Dry matter digestibility of the tall fescue hay used in this trial was determined in vivo, in vitro, and with the ratio technique using alkanes C_{29}, C_{31}, and C_{33} as internal markers corrected by their respective RR. The average values by method can be seen in Table 12 along with the comparisons between the actual in vivo and the other methods.

Table 12. Hay dry matter digestibility (DMD) obtained by different methods, and the comparisons between actual and estimated values.

Method	DMD, %	SEM (n) [a]
Actual	60.87	2.610 (42)
Estimated		
C29	55.54	2.640 (41)
C31	57.31	2.640 (41)
C33	57.40	2.640 (41)
IVDMD	64.16	2.609 (42)

[a] Standard error of the mean (number of observations).

Apparent in vivo DMD was slightly, although not significantly ($P \geq 0.161$), underestimated by using the natural odd chain alkanes C_{29}, C_{31}, and C_{33} and slightly overestimated ($P = 0.378$) by the IVDMD technique. Dove et al. (2002) found that the estimates obtained using C_{31}, C_{33}, and C_{35} were similar to the in vivo DMD, but contrary to our values, they found slightly higher estimated than actual in vivo values. The results reported by Dove et al. (2002) were based on two digestion trials conducted with sheep

fed ryegrass forage with a relatively high content of C_{35}. We worked with beef steers and tall fescue hay with very low content, sometimes negligible, of C_{35}. Therefore, in our estimations of DMD we included C_{29} instead of C_{35} as an internal marker. On the other hand, Sandberg et al. (2000), working with steers fed different types of forages, reported that n-alkanes based digestibilities were lower ($P < 0.01$) than in vivo digestibility for all diets. Likewise, Hendricksen et al (2002) tried different naturally occurring alkanes and found that, due to the great variation in fecal recoveries, C_{31} was the only one that estimated the group mean DMD with relative accuracy.

Estimations from the grab samples

The average DMFO and DMI found in this experiment with the in vivo and the marker method is shown in Table 13. Concentrations of Cr_2O_3 were determined in pooled samples collected from the bags or taken by rectum grab sampling twice per day. Alkanes are not included in these results because of a problem with the GC when running the grab samples.

Table 13. Actual and estimated dry matter fecal output (DMFO)
and intake (DMI) from chromic oxide concentration in feces.

Method or Collection period	DMFO, kg/d	DMI, kg/d
Actual	1.847	4.735
Total collection	1.850	4.732
Grab samples AM	1.846	4.720
Grab samples PM	1.846	4.717
P value [a]	0.999	0.999

[a] Observed significance level of the F test.

Both DMFO and DMI were accurately estimated without difference (P = 0.999) among sampling times (total collection, AM, or PM). Apparently the CRC released a constant amount of Cr_2O_3 during the day, confirming the theory of Parker (1989). Other researchers (Brandyberry et al., 1991; Hatfield et al., 1991; Luginbuhl et al., 1994) have also found a constant RR of Cr_2O_3 when dosed in CRC, but their estimations of DMFO were higher than the actual one due to the low recovery of the marker. Momont et al. (1994) applied the RR as correction factor in the calculations and they concluded that grab samples collected once daily on five consecutive days can be composited and used to reliably predict fecal output when Cr_2O_3 controlled release boluses are used. Also, in this trial, pooling the samples by steer across the collection period (7 d) improved the accuracy of DMFO and DMI estimations. According to Schneider and Flatt (1975), that happens because the day-to-day variations occurring during the collection period are removed from the analysis.

Chromic oxide has been widely used to estimate DMFO but its concentration in feces may present large variations (Le Du and Penning, 1982). Preliminary studies with CRC in sheep and cattle showed that intraruminal capsule technology has the potential to surpass existing methods of intake measurement because the uniform release of Cr_2O_3 into the rumen decreases diurnal variation of the marker in the feces and the single application of the CRC reduces both animal disturbance and labor requirements (Parker, 1989; Brandyberry et al., 1991, Momont et al., 1994).

Implications

Dry matter fecal output, intake and digestibility can be reliably estimated from digestion trials using chromic oxide and alkane controlled release capsules, provided an accurate recovery rate can be obtained for the markers. When hay dry matter digestibility was determined using natural alkanes as internal markers or in vitro techniques the values were slightly lower for the internal markers. However, when using the double alkane technique to estimate dry matter intake, only pairs of alkanes with similar recovery rates provided reliable estimates. Although, the capsules released a constant amount of

markers, fecal output and intake estimations improved when the samples were pooled over the collection period.

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CHAPTER V

Manuscript 3. Evaluation of the chromic oxide controlled release capsule technique in estimating dry matter intake of grazing steers

ABSTRACT. A digestion trial under grazing conditions was conducted at the Virginia Tech Kentland Farm to evaluate the use of a chromic oxide (Cr_2O_3) controlled release capsule (CRC) technique to estimate DM fecal output (DMFO) and voluntary intake. Six Angus crossbred steers (avg. BW 382 kg ± 16 kg), grazing a low endophite tall fescue pasture, were orally dosed with two intraruminal CRC on Aug 13, 2003. One capsule contained Cr_2O_3 and the other a mixture of dotriacontane (alkane C_{32}) and hexatriacontane (alkane C_{36}). The collection period started on Aug 21, 2003 and lasted for 7 d. During this time, feces were collected in bags and sampled twice per day at 0800 and 1700. Rectal grab samples were also collected in the morning and in the evening during the change of the bags. Forage mass was estimated with the plate meter at the beginning and at the end of the trial. Forage nutritive value was determined on samples collected daily from clipping and hand plucking techniques. Determinations included DM, ash, CP, NDF, ADF, and IVDMD, alkanes and Cr_2O_3. Data were analyzed with a repeated measure structure using the mixed procedure of SAS. Forage allowance was in between 12.48 and 14.81 kg of forage DM/100 kg of BW. Apparently, each of the CRC released a constant amount of Cr_2O_3, but the recovery rate was different ($P < 0.01$) among steers and extremely high (avg. = 1.892). However, when the recovery rate was used to correct the calculations of DMFO and DMI, estimated and actual values were not different ($P \geq 0.846$). Although the Cr_2O_3 fecal concentration and recovery rate in the AM and PM pooled grab samples were not different ($P \geq 0.219$) from the total collection (AM and PM), the DMFO and DMI estimates with the PM grab samples were different ($P \leq 0.04$) from the total collection method and AM sampling grab. It is suggested that in this kind of trials the estimated values of DMFO should be adjusted by marker recovery rate obtained by the total collection method in at least a small number of animals.

Key Words: Digestion Trial, Chromic Oxide, Steers, Tall Fescue, Dry Matter Intake.

Introduction

When animal performance cannot be measured, dry matter intake (DMI) and digestibility (DMD) probably represent the most useful and common indicators of the forage nutritive value and other indices of forage quality such as "the relative feed value" and "the relative forage quality" (Moore and Undersander, 2002). Unfortunately, under grazing conditions neither the DMI nor the DMD of the forage eaten can be measured. Nevertheless, they can be estimated if accurate measures of the dry matter fecal output (DMFO) and forage digestibility are possible (Van Soest, 1994). Feces can be collected in special bags or estimated by means of a marker. The marker is administered to the animal in a known quantity and its concentration is determined in feces in order to get an estimation of the DMFO. Dry matter digestibility is usually determined by in vitro or in situ procedures (Stern et al., 1997). An internal marker can also be used for this purpose.

Chromic oxide (Cr_2O_3) was introduced as an external marker by Edin in 1918 (Knapka et al., 1967) and since then it has been used extensively in digestion trials to estimate DMFO. Chromic oxide is usually coupled with the IVDMD in order to get an estimation of DMI in both ruminant and non-ruminant animals (Le Du and Penning, 1982). One of the problems with this method to estimate DMI in experiments with grazing animals is the possible diurnal variation of the marker concentration in feces, especially when Cr_2O_3 is dosed once or twice per day in gelatin capsules or paper tissue. Other methods of marker administration, such as the controlled release capsules (CRC) and the peristaltic pump, have been developed in order to minimize that problem and at the same time reduce the time, labor and stress caused when dosing animals with markers in the digestion trials (Parker et al., 1989). However, there is a question about the amount of marker being released by the capsules (Burns et al., 1994) and recovered in the feces. Therefore, the objectives of this trial were to:

1) Determine the recovery rate of Cr_2O_3 when dosed by means of the controlled release capsules to beef steers grazing a tall fescue pasture.

2) Compare the actual with the estimated fecal output, and calculate feed intake with the marker method.

Material and Methods

A digestion trial was conducted under grazing conditions to test the hypothesis of no difference in actual and estimated DMFO when estimates were made by the Cr_2O_3-IVDMD technique. Dry matter intake was calculated using both the actual and the estimated DMFO. The experiment was conducted at the Virginia Tech Kentland Farm, Blacksburg, VA. Six Angus crossbred steers (avg. BW 382 kg $\pm$ 16 kg) were allotted to a 0.607 ha paddock on Aug 18, 2003 and remained there until the end of the test. The pasture was an aftermath of low endophyte tall fescue that had been cut for hay. The steers had been grazing tall fescue pastures during the entire growing season of that year. They also had been previously trained to the use of harnesses and feces collection bags (Hanstings Canvas Manufacturing Co. NB, USA). On Aug 13, 7 d before the beginning of the collection period, each of the steers was dosed orally with two CRC, following recommendations made by the manufacturer (CAPTEC. Nufarm Lty., Auckland, NZ). One of the capsules contained Cr_2O_3 and the other contained a mix of n-dotriacontane (alkane C_{32}) and n-hexatriacontane (alkane C_{36}). The manufacturer's stated release rates were 1.5 g/d for a period of time between 22 to 31 d for the Cr_2O_3 CRC and 400 mg/d for a period of 20d for the alkanes CRC.

The collection period started on Aug 21, 2003 and lasted for 7 d. The feces collection bags had been fitted to the steers 2 d before the beginning of the collection period. During collection, feces were collected and sampled twice per day following the procedure given in Appendix VI. Briefly, the steers were walked from the paddock to the working facilities twice daily at 0730 and 1630. Each of the steers was restrained in the chute, the bag and its contents were removed and a clean empty bag was attached to the steer. The change of bags started at 0800 and 1700 and lasted for about 45 min. The bags were then transported to the feeding room on campus, the contents in each bag were weighed and mixed thoroughly, and a 5% sample was taken from each of the bags in the morning and in the evening. While sampling, the feces were placed in a double plastic

bag and the sealed bags were kept on ice within a cooler box. Immediately after finishing this activity, the samples were stored in a freezer at -18 ºC until analysis.

In addition, rectal grab samples of feces were collected from each of the steers in the mornings and evenings during the 7 d of the collection period. These samples were taken during the change of the feces collection bags, placed in sealed plastic bags and stored frozen (-18 ºC) pending analysis.

Forage DM mass was estimated once at the entrance of the animal to the paddock (Aug 18, 2003) and then again at the end of the trial (Aug 28, 2003) by the pasture plate meter method. The calibration equation for this double sample technique was built on five cut quadrats according to Haydock and Shaw (1975). The regression was applied on 30 observational quadrats in order to get a final estimation of forage mass. Dry matter content was determined by drying the samples in an a forced draft oven at 55 ºC for at least 72 h. Herbage nutrient content was determined using samples collected from the paddock every day of the collection period (7 d) by clipping and by hand-plucking. For both of the methods, one handful of herbage was collected every 20 steps when walking the paddock in an "X" pattern. When clipping, the herbage was cut with power driven hand clippers at about 2.5 cm above ground level, whereas for the hand-plucking method only the leafiest parts of an adjacent site were selected.

Laboratory procedures

All samples were handled and analyzed, in duplicate. The forage samples were also kept in the freezer (- 18 ºC) until ready for analysis. Then, they were freeze dried in a FreeZone 12 Liter lyophilizer (LABCONCO, Co. Kansas City, MO, USA) and ground in a Wiley mill (Thomas Wiley, Laboratory Mill Model 4, Arthur H. Thomas Co. Philadelphia, PA, USA.) to pass through a 1 mm screen. These samples were analyzed for DM, ash, NDF, ADF, and IVDMD. Alkanes, Cr_2O_3, and CP were determined in a subsample of this material that was ground to pass through a 0.5 mm sieve.

Fecal samples collected from the bags were thawed at room temperature for about 24 h. Then, the morning and evening samples of the same animal were mixed and two subsamples were taken. The first subsample, containing about 100 g of fresh material, was dried in an air-forced oven at 95 ºC for 48 h, ground in the Wiley mill to pass through a 1 mm screen and analyzed for DM, ash, NDF, ADF, and IVDMD. The second subsample, which contained about 20 g of fresh material, was placed in a beaker and cover with cheese cloth to be freeze dried. This dry subsample was ground to pass through a 0.5 mm screen and it was analyzed for DM, ash, CP, n-alkanes, and Cr_2O_3 content. The fecal grab samples were mixed by steer across the collection period (7 d), ending up with only one AM and one PM grab sample per steer. These samples were handled, freeze dried, and ground like the second fecal subsample from the bags. They were analyzed for alkanes and Cr_2O_3 content.

Dry matter and ash content were determined in ground samples from herbage and fecal samples following the AOAC (2000) procedures. Briefly, 0.5 g of the samples were placed in a crucible and dried for 24 h in a forced-draft oven at 100 ºC. Ash was determined in sequence leaving the samples in a muffle furnace for 3 h at 500 ºC. Crude protein (N X 6.25) was obtained by N content determinations of the samples in a PerkinElmer 2410 Nitrogen Analyzer (PerkinElmer Instruments LLC. Shelton, CT, USA) according with the AOAC (2000) procedure. The NDF and ADF determinations were made according to AOAC (2000) using the ANKOM 200/220 Fiber Analyzer following the manufacturer's procedure recommended in the operator's manual (ANKOM Technology, 1997a). The IVDMD was determined using the DAISY II[200/220] Rumen Incubator (ANKOM Technology, 1997b).

 Chromic oxide concentration in herbage and feces was determined according to the AOAC (2000) procedure with modifications. Wet ashing with nitric and perchloric acid of 0.5 g of ground samples (Muchovej et al., 1986) was followed by flame atomic absorption spectrophotometry in a PerkinElmer Analyst 800 (PerkinElmer Instruments, Uberlingen, Germany). Standard solution was prepared at 0.0, 1.0, 2.0, 3.0, and 4.0 ppm using a chromium reference solution (Fisher Scientific, NJ, USA).

Alkane extraction and analysis were conducted in the freeze-dried and ground subsamples following the procedure outlined by Mayes et al. (1986) with some modifications. Briefly, samples of feces and herbage weighing 0.1 and 0.3 g, respectively, were placed along with 0.0010 g of the internal standard (alkane C_{34}) in a 20 mL pyrex tube fitted with a screw cap. The samples were saponified with 7 mL of a 10% ethanolic KOH solution at 90 ºC for 3 h in a water bath with mixing of the contents every 30 min. After cooling, 7 mL of deionized water and 7 mL of heptane were added and the tube contents were mixed thoroughly. The organic extract was removed with a pipette, applied to a filtration column, and elute with about 10 mL of heptane into a 20 mL scintillation vial. Filtration columns were made of 5 mL disposable pipettes containing silica gel with glass wool stoppers. The eluent was then dried in a N evaporator unit (Organomation Associates, Inc. Berlin, MA, USA.). When ready for analysis, the residue was re-dissolved with 1 mL of heptane before injection of 0.5 µL into the capillary column of a GC (Agilent Technologies 6890. Santa Clara, CA) equipped with flame ionization detector, integrator, and a 7683 series autosampler. The column specifications were 30 m long, 0.52 mm i.d., and 1.5 µm of fused silica film thickness (Supelco Inc. Bellafonte, PA. USA). Oven temperature was programmed to hold at 240 ºC for 4 min, increase to 288 ºC at 3 ºC/min, and then increase to 298 ºC at 2 ºC/min. Helium was used as the carrier gas with a flow rate of 9.0 to 9.25 mL/min. The identity of alkanes was determined from their retention times relative to known standards, whereas peak areas were converted to amounts of alkanes in reference to the internal standard by the GC integrator.

Measured and estimated variables

All the calculations were made on DM basis with the following expressions:

1. Actual DM fecal output (DMFO, g/d) = morning plus evening collection of feces
    Note: 150 g were added per bag in compensation of the grab sample collected.

2. Calculated dry matter intake (DMI, g/d) = Actual DMFO / (1 - IVDMD)

3. Marker recovery rate (RR) = Marker in feces (g/d) / daily dose of marker (g/d)

4. Estimated DMFO with the use of external markers.

    DMFOE (g/d) = [Daily dose of marker (g/d) / marker concentration in feces (g/g DM)] X RR.

5. Estimated DMI with the use of external markers and the IVDMD.

    DMIE (g/d) = DMFOE (g/d) / (1 - IVDMD).

Alkanes were not used for any of these calculations because of a problem with the alkane program in the GC when running these samples.

Statistical analyses

Due to extreme variations in the daily Cr_2O_3 RR from steer ID No. 215, the statistical analyses were conducted using data from only the other five steers. This trial corresponded to a randomized complete block design with five replications. Steers were used as the experimental units, treatment was the method used to estimate the response variables (RR, DMFO, DMI), and days of the collection period (7 d) were considered repeated measures on time. Data were analyzed using the mixed procedure of SAS (2001) according to Littell et al. (2002). Linear contrasts were used to compare the actual vs. the estimated variables. Data from the grab samples were also analyzed with the mixed procedure of SAS but only included steers as experimental units and the total fecal collection, AM, and PM grab samples as the repeated measures. The SAS codes for these analyses are shown in appendix VII.

Results and Discussion

The estimated forage mass before and after the collection period was 5,592 and 4,711 kg/ha, respectively. The paddock size was 0.607 ha, the steers remained in the paddock for 10 d, and the average BW of the steers was 382 kg. Thus, the forage allowance was between 12.48 and 14.81 kg of forage DM/100 kg of BW. Therefore, forage allowance was not a restriction for the animals to express their potential voluntary feed intake. Actual DMFO per steer averaged 3.181 kg/d and the IVDMD was 75.83%, thus, the calculated mean DMI per steer was 13.205 kg/d. The nutrient content of the forage was higher in samples that were taken by hand plucking than in samples collected by the clipping technique (Table 14). It was assumed that the animals had opportunity to select, and ate leafy material more similar to the one collected with the hand plucking than with the clipping technique. The forage Cr_2O_3 concentration was negligible (0.13 mg/kg DM) and was not taken into account in any of the calculations.

Table 14. Least square means for the nutrient content of the forage
 (DM basis) sampled by clipping and by the hand plucking technique

| | Sampling technique | | |
Component	Clipping	Hand plucking	SEM [a]
	-----------------%----------------		
Ash	9.33	9.15	0.189
CP	10.61	14.57	0.317
NDF	53.69	44.97	0.553
ADF	29.79	25.67	0.324
IVDMD	66.83	75.77	0.772

[a] Standard Error of the mean (n = 7)

The ANOVA showed individual differences in Cr_2O_3 concentration (P < 0.001) and RR (P < 0.001) in feces. No effect was detected (P ≥ 0.671) due to day of collection period, which agrees with the reports of Parker et al. (1989), Brandyberry (1991) and Luginbuhl (1994). Apparently, the intraruminal CRC release a constant amount of Cr_2O_3.

The fact that the Cr_2O_3 RR was different among steers suggests that perhaps the release rate of the marker was different among individual CRC. Also in this trial, contrary to other reports (Hatfield et al, 1991; Buntix, 1992; Moshtaghi Nia and Wittenberg, 2002), the average Cr_2O_3 RR was unusually high (1.892). However, in agreement with our results, Momont et al. (1994) also found a RR that was constant throughout the collection period, without diurnal variations. In that experiment, the RR in the two of their trials was more than 11% greater than the release rate stated by the manufacturer for both sheep and cattle Cr_2O_3 CRC. In the present trial the mean RR was 89% higher than the release rate stated by the manufacturer. Furthermore, one of the steers (ID No. 215) had to be eliminated from the trial due to excessively high (3.453) and variable (± 1.648) RR. These results reinforce the idea that when dosing animals with Cr_2O_3 using intraruminal CRC, total fecal collections on a subset of the animals used in a study may be necessary to adjust marker estimates of fecal output (Hatfield et al., 1991). Based on the variability observed in their study, Brandyberry et al. (1991) stated that it would seem that five steers in a 7 d total fecal collection period would be required to estimate the release rate with an error of ± 5% of the mean.

When the RR was used as correction factor in the formula to estimate DMFO and DMI, there were no differences due to method (P ≥ 0.846), day of the collection period (P ≥ 0.583), or the interaction method by day (P ≥ 0.807) between actual and estimated values. The comparison between methods in estimating DMFO and DMI is shown in Table 15. In other studies to evaluate the use of Cr_2O_3 CRC, Pond et al. (1990), Buntix et al., (1992), and Luginbuhl et al. (1994) concluded that, although the RR seems to be constant during the test, in most of the trials the DMFO was overestimated as a consequence of a low recovery of the marker in feces. They did not use the RR in the formula to calculate DMFO and showed the problems in getting a reliable estimation of

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DMI when only the manufacturer's stated release rate is available. On the other hand, Momont et al. (1994) concluded that after verification of the manufacturer's stated Cr_2O_3 RR with a small number of animals, the technique has the potential to reduce animal handling, time, and disruption of animal grazing patterns compared with twice daily administration of Cr_2O_3 gelatin capsules or other dosing methods.

Table 15. Comparisons between actual and estimated
 dry matter fecal output (DMFO) and intake (DMI)

Method	DMFO	DMI
Actual, kg/ha	3.181	13.205 [a]
Cr_2O_3, kg/ha	3.207	13.314
P value [b]	0.846	0.848

[a] Calculated from actual fecal DM output and IVDMD.
[b] Significance level of the F test.

Estimates with the pooled samples

Marker concentrations and RR (values not shown) were not different ($P \geq 0.219$) in the pooled grab samples (AM and PM) than in the samples taken from total collection in bags. It means that there was no evidence of diurnal variation in Cr_2O_3 concentration in feces, which supports the results of other experiments with more intensive diurnal sampling (Hatfield et al., 1991; Luginbuhl, 1994; Momont et al., 1994). The DMFO and DMI estimated with the PM grab samples were different ($P \leq 0.04$) from the actual values (Table 16). These results are difficult to explain because all of the estimated values are coming from the same mathematical procedure. Likewise the estimated DMI is a value obtained using the same herbage indigestibility (1 - IVDMD) that was used for the total collection and AM, and PM grab samples. Thus, if there is no difference in the RR, the DMFO and DMI should be similar for the three kinds of sampling periods (total

collection, AM, and PM), which was not the case. Probably this is due to the slightly larger variation in the PM (1.92 ± 0.233) than in the AM (2.00 ± 0.137) sample mean. However, this result disagrees with those of Parker et al. (1989), Brandyberry et al. (1991) and Momont et al (1994).

Table 16. Comparison between actual and estimated dry matter fecal output
 (DMFO) and dry matter intake (DMI) with the pooled grab samples

Method of Estimation	DMFO, kg/d	DMI, kg/d
Actual	3.181	13.205 [a]
Estimated		
Total collection	3.208	13.314 [b]
Grab AM	3.125	12.930 [b]
Grab PM	3.557 [c]	14.718 [c]
SEM [d]	0.117	0.483

[a] Calculated from actual DM fecal output and IVDMD.

[b] Calculated from DM fecal output estimated with Cr_3O_2 and IVDMD.

[c] Different ($P < 0.05$) from actual.

[d] Standard error of the mean.

Implications

In grazing trials where forage allowance is not a restriction of the voluntary feed intake, it is likely that the quality of the forage consumed will be better estimated by hand plucking than by clipped samples. Although the chromic oxide recovery rate from the controlled release capsules seems to be constant during the collection period, there may be variations in the amount of the marker being released among different capsules. Thus, when fecal excretion and DM intake are to be calculated in a digestion trial using the

chromic oxide controlled release capsules, the release rate needs to be verified by total collection, at least in a few animals. Only morning or evening collection of rectal grab samples do not assure reliable estimates of DMI even when there is no evidence of diurnal variation of marker concentration in feces. Again, it would be recommended to adjust these values with those from total collection of feces.

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CHAPTER VI

General Discussion and Implications

Agricultural commodities have to be produced in quantity and quality to satisfy the food necessities of a growing world population. This mission has been mostly accomplished in the developed countries, but still there are many things to do in the rest of the world. In modern agriculture, the improvements in crop production have been achieved due to the correct application of better methods and technologies in many aspects of the production systems. Those "new" methods should be evaluated and their effects should be measured before their release for commercial applications. However, direct measures are not always possible, and hence, the researchers have to devise methods to estimate the outcome of interest, commonly the crop yield and quality.

In forage evaluation and grazing studies, herbage mass is used as the base measurement for the calculations of other important grassland attributes such as crop growth rate, utilization, and decomposition. Dry matter intake (DMI) and dry matter digestibility (DMD) probably represent the most common indicators of forage quality. Unfortunately, those measurements are impractical and sometimes impossible to obtain in grazing trials. Therefore, this study was conducted to investigate: 1) the use of direct and indirect methods to estimate forage mass on a tall fescue (*Festuca arundinacea*, Schreb) pasture, and 2) the use of chromic oxide and alkanes controlled release capsules to estimate dry matter fecal output (DMFO), DMI, and DMD in beef steers under indoor and grazing conditions.

In the first trial, forage mass estimate from the direct herbage cutting method (HCM) was compared with three indirect methods, the pasture capacitance meter (PCM), the pasture plate meter (PPM), and the visual estimation (VE). Data from the sampling date of Aug. 8, 2002 are not included because the lack of forage in the pastures and humidity in the soil affected the results of the indirect methods, especially, the PCM. The results from 2 yr showed that the coefficients of determination (r^2) for the calibration equations of the indirect methods were highest for the VE (from 0.879 to 0.942),

intermediate for the PPM (0.816 to 0.931), and lowest for the PCM (0.726 to 0.898). These r^2 values are better than those reported by Bransby et al. (1977), Murphy et al. (1995), and Rayburn and Rayburn (1998) and they are comparable with those found by Stockdale and Kelly (1984), Griggs and Stringer (1988), and Harmoney et al. (1997).

Overall there were differences due to sampling method ($P < 0.01$), sampling date, and the interaction method by day in forage mass estimates. Differences in sampling dates were expected because the paddocks were sampled at different points in time during 2002 and 2003, and most likely, the forage mass changes throughout the growing season. The working hypothesis was that methods should not show differences in forage mass estimates, which was not the case. Moreover, linear contrast showed that the value of forage mass obtained with the HCM was overestimated ($P < 0.01$) by each of the three double sampling techniques. Most researchers have evaluated the methods based only on the accuracy of the calibration equations, without the fully and independent application of the procedure as was made in this trial. Michalk and Herbert (1977), and Murphy et al. (1995), and Correll et al. (2003) seem to favor the use of the double sampling technique, while the opinion of Gabriels and Van Den Berg (1993), and Sanderson et al. (2001) is that at present, it is impossible to make an accurate prediction of the pasture DM yield based on the use of indirect methods. Meanwhile, we only can conclude that the HCM in this experiment gave estimates consistently lower than the three indirect methods. However, the accuracy of these techniques to estimate the actual forage mass remains to be elucidated, preferably by comparisons with the harvest of the whole plots.

The second experiment was a digestion trial conducted indoors at the Virginia Tech Smithfield Barn to determine the recovery rates (RR) of Cr_2O_3 and alkanes C_{32} and C_{36} when dosed in intraruminal controlled release capsules (CRC). Dry matter intake and digestibility were also determined in beef steers fed tall fescue hay. During the 7 d of the collection period, the actual DMI, DMFO, and DMD were 4.735 kg/d, 1.847 kg/d, and 61%, respectively. Alkane C_{31} was found in higher concentration than any other alkane in hay (143 mg/kg) and feces (373 mg/kg), which agrees with other reports (Piasentier et al., 1995; Boadi et al., 2002; Bugalho et al, 2004). No differences were found in the RR of

either alkanes ($P \geq 0.106$) or Cr_2O_3 ($P = 0.341$) due to sampling day. These results support the hypothesis suggested by Parker et al. (1990), Hatfield et al. (1991), and Dove et al. (2002) that the CRC released a constant amount of marker during the collection period. When DMFO, and DMI were estimated with Cr_2O_3, and alkanes C_{32} and C_{36} as external markers, and the calculations were adjusted by the respective RR, there were no differences between these and the actual measured values ($P \geq 0.813$). Estimates with Cr_2O_3 reflect better accuracy with an overestimation of less than 2% of the actual DMI. Momont et al. (1994) also reported a similar difference between the actual and estimated DMFO, and DMI.

When using the double alkane technique to estimate DMI as proposed by Mayes et al. (1986), the only pairs of alkanes that gave reliable estimate were the C_{32}/C_{31}, and C_{36}/C_{31}. These results do not agree with the general recommendation of using the alkanes C_{32}/C_{33} reported by others (Mayes et al., 1995; Berry et al, 2000; Dove et al., 2002). That is likely due to the different RR found in this trial for alkane C_{33} (1.30) in comparison with alkanes C_{31} (1.09), C_{32} (0.81) and C_{36} (1.00). It is a prerequisite of this technique that the pair of alkanes used in the fecal ratio have similar RR (Mayes et al. 1986). Estimates of DMFO and DMI using data from only the concentration of Cr_2O_3 in the pooled grab samples were similar ($P \geq 0.99$) to the actual values regardless of the type of sample (total collection, AM, or PM), provided the calculations were adjusted for the respective RR.

The third experiment was a digestion trial conducted under grazing conditions at the Virginia Tech Kentland Farm. The objective was to evaluate the use of the Cr_2O_3 CRC technique to estimate DMFO and DMI in beef steers. Forage allowance was in between 12.48 and 14.81 kg of forage DM/100 kg BW. Thus, forage mass was not a restriction of voluntary feed intake. There were differences ($P < 0.01$) in Cr_2O_3 concentration and RR, but no effect was detected ($P \geq 0.67$) due to day of collection, which agrees with the results of Parker et al. (1989), Brandyberry et al. (1991), and Luginbuhl et al. (1994). Apparently, the CRC released a constant amount of Cr_2O_3, but the fact that the marker RR was different among steers suggests that the release rate from

individual CRC may be different. Nevertheless, when the RR was used as correction factor in the formula to estimate DMFO and DMI, estimated values were similar ($P \geq$ 0.846) to the actual ones. In other studies to evaluate the use of the Cr_2O_3 CRC technique in different conditions, Pond et al. (1990), Buntix et al. (1992), and Luginbuhl et al. (1994) concluded that although the RR seems to be constant during the test, in most of the trials the DMFO was overestimated as a consequence of a low RR of the marker. These authors did not adjust the calculations for the RR. Marker concentration and RR were not different ($P \geq 0.219$) in the pooled grab samples (AM and PM) than in the samples taken from total collection of feces. It means that there was no evidence of diurnal variation in the Cr_2O_3 RR in this trial, which supports the results of other experiments with more intensive diurnal sampling such as the ones reported by Hatfield et al. (1991), Luginbuhl et al. (1994), and Momont et al. (1994).

Some implications can be drawn from these trials. When estimating the forage mass of a pasture, it is important to select the most suitable method and then, pay attention to the sampling procedure because different techniques may give different estimates of the same actual value. Sampling intensity, size, and shape of the quadrat play an important part in the direct methods. Accuracy of the calibration equations and sample size are key elements when using a double sampling technique. In digestion trials with beef steers, dry matter fecal output, intake, and digestibility can be reliably estimated using chromic oxide and alkanes C_{32} and C_{36} as external markers dosed by means of intraruminal controlled release capsules. However, the marker recovery rates have to be determined and used to correct the calculations. If dry matter intake is to be estimated with the double alkane ratio technique, it is important that the pair of alkanes used in the formula have similar recovery rates. Finally, it seems that the estimations of fecal output and feed intake are more accurate when the samples are pooled by individual animals across the collection period, compared to the estimations using the daily samples.

CHAPTER VII
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Appendices

Appendix I. Statistical models and SAS codes used to build the calibration equations and compare the methods in estimating forage mass of the tall fescue pastures.

All the calibration equations were calculated using the regression procedure of SAS version 8.2 (SAS Inst. Inc., Cary, NC) with the simple linear regression model:

$Y_i = \beta_0 + \beta_1 X_i + \varepsilon_i$

Where

Y = Actual dry matter yield (kg/ha)

X = The indirect method record (IM = PCM, PPM, VE)

β_0 = The regression line intercept

β_1 = The slope of the regression's line or regression coefficient

ε_I = Experimental error.

The SAS code used was:

proc reg data = DM yield;

model actual DM = indirect method (IM)/p; (IM = PCM, PPM, VE)

run;

In order to compare the slopes of the regression's lines for methods and methods across sampling dates, the working hypothesis of equality was tested using the GLM procedure following model:

$Y_{ijkl} = \mu_0 + \alpha_i + \varphi(\alpha_i)_j + \gamma_k + \beta_j X_{ijkl} + \beta_{jk} X_{ijkl} + \varepsilon_{ijkl}$

Where

Y_{ijkl} = DM yield with the herbage cutting method

μ_0 = General mean

α_i = Year effect (i = 1, 2)

$\varphi(\alpha_i)_j$ = Sampling date within year effect (j = 1, 2, 3, 4)

γ_k = Forage mass predicted with the indirect methods (k= 1, 2, 3)

β_j = The slope of sampling dates

X_{ijkl} = The l-th observation with the k-th method on the j-th date and the i-th year

β_{jk} = The slopes of methods

ε_{ijkl} = Experimental error

The SAS code for this model was:

proc glm data = DM yield;

class year date indirect method (IM = PCM, PDM, VE);

model HCM = year date(year) IM DMpredicted IM*DMpredicted

 IM*DMpredicted*date(year) / solution;

run;

For the whole period of the study and for years ANOVA were carried out with the mixed procedure to test the null hypothesis of no difference among the methods in DM yield estimation. The model included only the fixed effects of paddock as block, method as treatment, date as repeated measure and the interaction method by date. The SAS code was as follows:

Proc mixed data = foragemass;

Class paddock method date;

Model DMyield = paddock method date method*date / ddfm = satterth;

Repeated date / type = ar(1) subject = paddock(method) r rcorr;

Run;

Individual contrasts were used to compare the mean DM yield estimated with the HCM method with the mean DM yield estimated with each of the three indirect methods. Year 2002 was also contrasted with yr 2003. The SAS codes for these contrasts were:

Contrast 'HCM vs PCM' method +1 -1 0 0;

Contrast 'HCM vs PPM' method +1 0 -1 0;

Contrast 'HCM vs VE' method   +1 0 0 -1;

Contrast '2002 vs 2003' date +1 +1 +1 +1 -1 -1 -1 -1;

Run;

Appendix II. Calibration equations for the pasture capacitance meter by sampling date

Sampling dates	Regression***				DM mass, kg/ha [c]	RSD, kg/ha [d]
	B_0	B_1	$SE(\beta_1)$ [a]	r^2 adj [b]		
June 13, 2002	-1095.62	1.75	0.175	0.772	2712	859
Aug. 8, 2002	-1323.69	1.88	0.350	0.491	2230	1292
Sep. 5, 2002	-1460.99	3.02	0.342	0.726	1907	877
Oct. 17, 2002	-1956.18	1.80	0.163	0.807	2173	597
June 5, 2003	17.87	1.20	0.085	0.872	3471	816
July 17, 2003	125.28	1.60	0.125	0.850	3585	971
Sep. 25, 2003	-1369.45	2.03	0.169	0.832	4533	1148
Nov. 10, 2003	-1045.06	2.62	0.163	0.898	5285	983

*** All regressions ($Yi = \beta_0 + \beta_1 X + Ei$) were significant ($P < 0.001$).

[a] Standard error of the regression coefficient (β_1).

[b] Adjusted coefficient of determination.

[c] Mean dry matter mass estimated from the cut quadrats.

[d] Residual standard deviation from the regression, $n = 30$.

Appendix III. Calibration equations for the pasture plate meter by sampling date

| Sampling dates | Regression*** | | | | DM mass, | RSD |
	B_0	B_1	$SE(\beta_1)$ [a]	r^2 adj [b]	kg/ha [c]	kg/ha [d]
June 13, 2002	-300.97	219.97	19.344	0.816	2712	773
Aug. 8, 2002	-830.25	260.84	39.115	0.600	2230	1146
Sep. 5, 2002	-1452.21	399.87	31.741	0.845	1907	660
Oct. 17, 2002	-683.21	287.58	23.128	0.841	2173	541
June 5, 2003	-420.71	213.64	15.168	0.872	3471	816
July 17, 2003	-1044.23	332.27	16.734	0.931	3585	658
Sep. 25, 2003	-2218.62	429.60	30.340	0.873	4533	999
Nov. 10, 2003	-2211.98	459.02	28.643	0.898	5285	985

*** All regressions ($Y_i = \beta_0 + \beta_1 X + E_i$) were significant (P < 0.001).

[a] Standard error of the regression coefficient (β_1).

[b] Adjusted coefficient of determination.

[c] Mean dry matter mass estimated from the cut quadrats.

[d] Residual standard deviation from the regression, n = 30.

Appendix IV. Calibration equations for the visual estimation method by sampling date

| Sampling dates | Regression*** | | | | DM mass, | RSD, |
	B_0	B_1	$SE(\beta_1)$ [a]	r^2 adj [b]	kg/ha [c]	kg/ha [d]
June 13, 2002	126.54	1.60	0.110	0.879	2713	627
Aug. 8, 2002	-125.19	2.26	0.212	0.795	2230	820
Sep. 5, 2002	-201.97	2.31	0.129	0.917	1907	483
Oct. 17, 2002	113.51	1.477	0.094	0.894	2173	442
June 5, 2003	104.55	1.43	0.079	0.918	3471	653
July 17, 2003	-165.21	2.09	0.133	0.895	3585	815
Sep 25, 2003	-375.47	1.81	0.111	0.901	4533	884
Nov 10, 2003	-294.73	2.28	0.105	0.942	5285	740

*** All regressions ($Yi = \beta_0 + \beta_1 X + Ei$) were significant ($P < 0.001$).

[a] Standard error of the regression coefficient (β_1).

[b] Adjusted coefficient of determination.

[c] Mean dry matter mass estimated from the cut quadrats.

[d] Residual standard deviation from the regression, $n = 30$.

Appendix V. Forage mass estimates with four methods during 2002 and 2003

Sampling methods	2002		2003	
	DM mass, kg/ha	Contrast P-val [a]	DM mass, kg/ha	Contrast P value [a]
Herbage cutting method	1383	------	2523	------
Pasture capacitance meter	1551	0.095	3909	< 0.001
Pasture plate meter	1659	0.007	4065	< 0.001
Visual estimation	1638	0.013	3740	< 0.001
SEM [b]	69		136	

[a] Observed difference for the contrasts of HCM with each of the indirect methods.

[b] Standard error of the mean by method, n = 24.

Appendix VI. Procedure followed in collecting, weighing, and sampling feces during the digestibility trials using steers fitted with fecal collection bags

General

    A. Used pre-tamed steers.

    B. Used two bags per steer and kept one or two spare bags.

    C. Identified and tared all the bags.

    D. Practiced the procedure during the adaptation period.

    E. Set the schedule for sampling, feeding, and any other management.

    F. Kept the environment as constant and quiet as possible.

    G. Fitted the harnesses to the steers and attached the bags to the harnesses at least 4 d before the beginning of the collection period.

    H. The feces were collected twice per day.

    I. Any sample and sub-sample were labeled and recorded in data sheets.

Collecting and Sampling Feces

A. Obtained a tare weight for each of the feces collection bags.

A. Restrained the steer within in the squeeze chute.

B. Released the bag with its content from the harness and immediately attached the other empty, clean bag.

C. Placed the bag in a safe place and repeated the process with the rest of the steers in the trial.

D. Took the bags with its content to the feed room.

E. Weighed the bags (one at a time) with its content and recorded the weight in the data sheet.

F. Emptied the feces of one bag into the bowl of the mixer.

G. Mixed the feces thoroughly by hand.

H. Took a 5% sub-sample from several spots on bowl and placed them in a labeled double plastic bag.

I. Emptied, cleaned, rinsed, and dried the bowl.

J. Repeated the process for each of the feces collection bags.

K. Immediately, these sub-samples were put in the freezer, within a cardboard box identified with the title of the trial and the responsible persons.

L. Washed and dried the bags in the washer and dryer machine, respectively.

M. Weighed the dried bags and recorded the weight in the respective data sheet.

Appendix VII. SAS codes for the digestibility trials

       A. SAS code for the analysis of the data from total feces collection in a complete randomized block design with six steers as blocks (EU), method (actual and estimated) as treatment, and day (7) of the collection period as repeated measure on time. The response variables were marker's recovery rate, DM fecal output, intake, and digestibility.

```
Data fecal;
Input steer $ method $ day $ var;
Datalines;
Proc print;
Proc mixed data = fecal;
Class steer method day;
Model var = steer method day method*day/ddfm = satterth;
Repeated day/type = ar(1) subject = steer(method) r rcorr;
Lsmeans method day method*day;
Contrast 'Actual vs. method1' method +1 -1 0 0;
Contrast 'Actual vs. method2' method +1 0 -1 0;
Contrast 'Actual vs. method3' method +1 0 0 -1;
Run;
```

AppendixVII. SAS codes for the digestibility trials (Contd.)

B. SAS code for the analysis of the data from grab samples in a complete randomized block design with six steers as blocks (EU) and time period (total, AM, and PM) as repeated measure. The response variables were Cr_2O_3 concentration in feces, recovery rate, fecal output, and dry matter intake.

Data grabs;

Input steer $ period $ vars;

Datalines;

Proc print;

Proc mixed data = grabs;

Class steer period;

Model var = period;

Repeated period/type = ar(1) r rcorr subject = steer;

Lsmeans period;

Contrast 'Actual vs. AM' period +1 -1 0 0;

Contrast 'Actual vs. PM' period +1 0 -1 0;

Contrast 'Actual vs. TOT.' period +1 0 0 -1;

Run;

VITA

Isaias Lopez-Guerrero son of Josefina Guerrero Herrera and Francisco Lopez Guerrero (deceased) was born in Jesús del Monte, State of Guanajuato, Mexico on July 6th, 1961. He attended elementary school, middle school, and high school at Leon city. In 1984, he graduated from the Agrarian Autonomous University, Antonio Narro. Isaias obtained his Master of Science degree in Animal Production, oriented toward Forages from the Autonomous University Chapingo at Chapingo, Mexico (1988 to 1990). He has attended several actualization courses, including a multidisciplinary research course for forage production at the International Center for Tropical Agriculture in Colombia from February 2nd to August 29th, 1987. Isaias has been working for the National Institute of Forestry, Agriculture and Animal Research (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias –INIFAP-) at the Department of Forages since 1985 as a scientist-extensionist titular researcher at "La Posta" research Center, in Paso del Toro, Veracruz, Mexico. Isaias Lopez has participated as author or coauthor in 15 abstracts and 21 papers. He is author of one chapter in a book about the management of the dual purpose cattle in the tropical regions of Mexico, and he has been part of the advisory committee for eight undergraduate theses. He is married to Teresa Beatriz Garcia-Peniche since 1990, and they have three children: Tania Beatriz, Isaias Jr, and Teresa Nathalia.