Review of Literature

More precise and accurate methods are needed for estimating fecal kinetics, particularly, fecal output (FO, kg/d DM), the prefecal mass (PFM, kg) and its turnover time (TT, d), and marker methods are being developed for this purpose (Blaxter et al., 1956; Haenlein et al., 1966; Dove and Mayes, 1996; Holland et al., 1998). These variables are important in studies of feed intake, digestibility, water balance, power:weight ratio, exercise performance, certain digestive disorders and environmental impact of grazing animals (Martin et al., 1989; Oldham and Tamminga, 1995; Kronfeld, 1996). Pasture composition is variable, and supplementation is usually needed to provide optimal nutrition for the horse. For ration evaluation of grazing animals, daily intake (DMI, kg/d DM), proximate and chemical analysis, botanical composition, FO and digestibility (DMD, % DM) are needed.

Nutritional experiments on grazing horses require estimates of individual intakes of pasture or supplement for the horse to be used as the statistical unit, otherwise the plot or paddock may appear as the statistical unit, as in agronomy (Snedecor and Cochran, 1967). Current methods for estimating the daily intake of energy and nutrients of grazing horses are imprecise because of the difficulties associated with measuring pasture consumption directly. Estimates of DMI of pasture in horses are routinely obtained using the ‘by difference’ method, where estimates of supplementary hay and/or concentrate are subtracted from the National Research Council (NRC 1989) estimates of mean intake of DMD, or digestible energy (DE). These estimates are based on the body weight and physiological condition (maintenance, pregnancy lactation, growth and work) of the horse (Pagan, 1995; Kronfeld, 1998). Any difference in DMD or DE is assumed to be provided by the pasture DMI, so this estimate of DMI accumulates multiple errors. In practice, negative values have often been calculated for horses observed to graze. Recognition of the inadequate accuracy and precision of the ‘by difference’ method has prompted the development of indirect methods using markers for estimating pasture DMI of ruminants (Kotb and Luckey, 1972; Krysl et al., 1988; Owens and Hanson, 1992), and other herbivores (Martin et al., 1989; Mayes et al., 1995).
Conventional digestibility trials and metabolic balance experiments use total collection (TC) measurements to determine estimates of FO and apparent DMD (Van Dyne, 1969; Sutton et al., 1977; Holechek et al., 1986). Apparent DMD of forage is the proportional difference between quantities of DMI and FO (Minson, 1990). It is calculated from the weights of food offered and food refused (orts), and the weight of feces collected in a tray or harness. The TC method is labor intensive and expensive, which restricts the number of animals. Undercollection of feces or orts leads to overestimation of DMD. Also, stall confinement may disrupt feeding behavior. This method for estimating DMI, DMD and FO, however, usually serves as the standard to validate other methods.

Indirect methods of estimating DMD employ markers or sacs in vivo, and enzymes or microbial fermentation in vitro. In vitro methods may be calibrated with forage samples of known in vivo DMD (Minson, 1990). Ground forage samples are fermented with microorganisms in a buffered medium under controlled conditions of anaerobiosis, temperature, and pH (Tilley and Terry, 1963; Minson, 1990). Estimates of DMD in vivo correlated with estimates of DMD in vitro in a 48 h digestion period with whole rumen fluid, followed by a 24 h digestion with pepsin-HCL (Tilley and Terry, 1963). An in vitro technique adapted from Tiller and Terry (1963) used cecal fluid of horses to estimate DMD of equine feeds (Trevor-Jones et al., 1991). Differences in digestive efficiency within and between animals due to level of intake or physiological state often complicate in vitro methods (Orton et al., 1985; Martin et al., 1989).

Digestibility has been predicted from feed contents of crude fiber and more recently neutral detergent fiber (NDF) and acid detergent fiber (ADF). It has also been predicted from infra-red spectra (NIRS) of feed fed to ruminants (Minson, 1990). Similarly, NIRS has been used to predict digestible organic matter (DOM) in horses (Andrieu and Martin-Rosset, 1995). Predictions of DDM and DOM have been approximately twice as precise from NIRS ($R^2 > .80$) than from crude fiber (Givens et al., 1997). It is recommended, however, that NIRS should only be used to predict the nutritive value of forage of the type that is used to calibrate the instrument. Hence, NIR may be used more confidently to predict in vitro digestibility but not in vivo digestibility (Martens and Naest, 1987). Additional methods of
estimating forage DMD include regressions relating forage DMD to fecal composition, and the use of indigestible internal markers (Minson, 1990).

**Markers in Ruminant and Equine Nutrition**

Since direct measurements of pasture intake, diet composition and nutrient utilization are difficult in the field, indirect methods using internal and external markers have been developed (Sutton et al., 1977; Owens and Hanson, 1992; Mayes et al., 1995). Internal markers are natural constituents of the plant, and are used to estimate DMD. External markers are substances administered to the animal and used to estimate FO. Pasture DMI can then be calculated from the marker estimates DMD and FO. For a substance to qualify as a marker in nutritional studies, it should have these characteristics: be inert with no toxic, physiological or psychological effects; be indigestible and unabsorbable in the digestive system; have physical similarities to the material it is to mark; be intimately mixed with, and remain uniformly distributed in the digesta; have no influence on, or be influenced by the digestive tract or its microbial population; pass through the tract at a uniform rate, and have a specific and sensitive method of estimation (Koth and Luckey, 1972; Maynard et al., 1979; Owens and Hanson, 1992).

**Marker Dosing Methods**

All marker procedures use one of two types of dosing and one of two types of sampling. 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 a plateau (Pond et al., 1988). Subsequently, digesta from specific sites, and/or fecal grab samples are collected at successive times (Owens and Hanson, 1992). Pulse-dosing typically is used to estimate digesta volume and retention times in specific parts of the gastro-intestinal tract (GIT). Flow rate can then be calculated from estimates of the volume and retention time (Pond et al., 1988). Continuous dosing is used primarily to measure instantaneous flow at a specific point in the digestive tract, e.g. duodenal flow and FO. Continued sample collection after administration of a continuous marker enables fecal kinetics to be examined (Faichney, 1980).
Verification of Marker Results

Marker estimates of FO can be verified by comparison with TC of feces (Krysl et al., 1985, 1988; Musimba et al., 1987). In addition, a method to determine whether markers are functional and if sampling is representative when markers are fed continuously, is to compare the ratio of two markers in digesta samples with the ratio being fed (Faichney, 1980; Faichney et al., 1980; Ortigues et al., 1990). When conditions are steady state, the ratio of concentrations of two markers at any point in the digestive tract equals the ratio being fed. The flow of digesta has been differentiated into a particulate and fluid phase system (Faichney, 1975), but this partition is an approximation because particles of different sizes and fluid at different sites probably flow and mix at different rates. With sheep receiving continuous feeding and continuous marker infusion, the ratio of $^{51}$Cr-EDTA, a marker for fluids, to $^{103}$Ruthenium-phenanthroline (Ru-P), a marker that associates with particles, in abomasal samples matched the ratio being infused. However, when sheep were fed once daily, but markers were infused continuously, the ratio of Cr:Ru-P in abomasal digesta varied from the ratio being infused (Faichney, 1975, 1980).

Internal markers

A variety of plant components have been used as internal markers in digestion trials. Lignin was the first, but it can be digested slightly and variably by fungi in the rumen and large bowel, and its proximate analysis is difficult and inaccurate (Elam and Davis, 1961). Acid insoluble ash (AIA) has been used as an internal marker in digestibility studies. Estimates of DMD by AIA and TC were similar in the rabbit but not the horse (Schurg et al., 1977). In a subsequent study, Schurg (1981) compared the TC method to chromic oxide, AIA, and lignin markers. There were no differences in TC, chromic oxide or AIA results, but recovery of lignin was underestimated between 12 and 18 % and considered unreliable. In stall-fed horses, AIA consistently underestimated DMD compared to TC procedures, however, differences were not significant (Sutton et al., 1977; Orton et al., 1985; Cuddeford and Hughes, 1990). To estimate digestibility, the marker must be mixed thoroughly through the chyme in the small intestine. Chromic oxide, for example, should be mordanted to forage to estimate digestibility (Cuddeford and Hughes, 1990), but it may be given as a
bolus to estimate FO (Haenlein et al., 1966; Schurg, 1981; Barbisan et al., 1993; Holland et al., 1998).

Apparent DMD estimates of a mollassed, chaffed grass hay/straw mixture was determined using Cr-mordanted hay (Cuddeford and Hughes, 1990). Mean Cr recovery was 96.5%. Comparisons were made with AIA, and no differences were found between the 2 marker methods. However, Cr measurements consistently underestimated DMD values and AIA consistently overestimated DMD values measured by TC. Large variations in fecal Cr concentration were observed over 24 h for each horse. Parkins et al. (1982) also reported a large degree of Cr concentration diurnal variation in horses. Frape (1982) reported that values of DMD derived by AIA were higher than obtained using Cr, however, the two indicator methods were compared with each other but not TC, which may limit the usefulness of the results. The use of Cr as a fecal marker led to lower DMD values than those obtained by TC in horses (Sutton et al., 1977; Orton et al., 1985).

In recent years, alkanes have been used as internal markers for estimating pasture DMI in ruminants (Mayes et al., 1986; Dove and Mayes, 1991; Mayes et al., 1995). Alkanes are hydrocarbons in the cuticular wax of plants. Alkanes usually occur as mixtures and range in chain length from C21 to C37. The highest concentration of alkanes (> 90%) in most plants have odd numbers of carbon atoms, and C29, C31 and C33 usually have the highest concentrations in herbage (Dove and Mayes, 1991). Alkane fecal recoveries observed in sheep, goats and cattle increased with increasing chain length (Mayes and Lamb, 1984; Dove and Mayes, 1991). More recent research has suggested that alkane recoveries may not be affected by increasing carbon chain lengths in non-ruminants (Dove and Mayes, 1996). Companion observations on alkanes in the present experiments confirm similar recoveries of alkanes for C27 to C 35 in horses (Ordakowski, 1998). Fecal recoveries of odd-chain alkanes did not increase with increasing carbon chain length in studies with horses, ponies and pigs (Mayes et al., 1995).
**Rare-Earth Markers**

Rare-earth elements are indigestible, become tightly bound with plant material, flow through the digestive tract in close association with indigestible feed residues, and have been used as internal markers to estimate DMD (Ellis, 1968), and rate of passage (Uden et al., 1980). An advantage of using rare-earth markers is that multiple markers may be used simultaneously while studying particle digestion and flow (Turnbull and Thomas, 1987). Four rumen-cannulated steers were used to compare pulse-dosed Ru-P with dysprosium-(Dy) and ytterbium (Yb)-labeled hay for particulate rate of passage estimates (Goetsch and Galyean, 1983). Fecal recoveries were evaluated in a two-compartment model to calculate rate constants for ruminal mixing of newly ingested particles ($k_1$) and entry of small particles into the rumen small particle pool ($k_2$). Estimates for $k_1$ did not differ ($P > .05$) among the rare-earth markers, however, $k_2$ estimates were less ($P < .05$) for Ru-P (2.06 %/h) than for Dy- (4.60 %/h) and Yb-labeled hay (4.44 %/h). Total mean retention time was greater ($P < .05$) with Ru-P (60.4 h) than with Dy- or Yb-labeled hays (average 42.1 h). The study showed that pulse-dosing with Ru-P may result in non-specific labeling of rumen particulates and yield passage rate estimates different from those obtained with labeled hays.

A study to compare the passage of fiber labeled by mordanting with a rare-earth element was conducted using cannulated cows and steers (Pond et al., 1988). Coastal bermudagrass hay was labeled with Cr by the Cr-mordant procedure and with $^{177}$Lutetium ($^{177}$Lu) applied to the same fiber. Neutral detergent fiber prepared from the same hay was labeled with Yb, $^{169}$Yb, terbium (Tb) and $^{160}$Tb by soaking overnight followed by thorough washing and drying. The bermudagrass fiber labeled with $^{160}$Tb was administered at the beginning of a meal, and the fiber labeled with Tb was administered at the end of a meal to determine whether dosing time relative to consumption of a meal could affect the rate of passage estimates within the same animal. The effects of time of marker administration on passage kinetics (markers administered in 24 h intervals) were evaluated with $^{169}$Yb (h 0) and Yb- (h 24) labeled bermudagrass. The effects of time of marker administration on passage kinetics when given before or after a meal was evaluated with $^{160}$Tb (h 12) and Tb- (h 15) labeled bermudagrass.
The use of radioisotopes resulted in much lower mg marker/g of fiber compared with the stable markers and was thought to reduce the problems associated with exceeding the binding capacity of the fiber and in altering the specific gravity of the marked fiber. In addition, by using the stable and radioisotope forms of Yb, estimates of passage characteristics at different times could be made. Passage rates were not different \((P > .05)\) within fiber source for rare-earth passage. There was also no difference between the passage characteristics of Cr-mordant and \(^{177}\text{Lu}\). However, passage rate of particles administered at the beginning of the meal \((^{160}\text{Tb})\) was 42 % higher than for particles at the end of the meal \((\text{Tb})\). The results showed that the flow characteristics of rare-earths applied by the soak and rinse procedure were the same as those of the more strongly bound Cr-mordanted fiber. In addition, although animals in a steady-state condition seem to have similar passage characteristics when markers are dosed on consecutive days, care must be used to avoid dosing with markers at different times relative to meal consumption by the animal (Pond et al., 1988).

\textit{\(^{89}\text{Yttrium}\).} A ruminal-cannulated steer was used to simultaneously determine ruminal liquid turnover and particle turnover rates of five particle sizes of corn obtained from two processing methods, and marked with rare-earth metals (Turnbull and Thomas, 1987). Particle sizes of steam-rolled corn (SRC) and cracked corn (CC) were separated by dry-sieving. The SRC particles, 4 mm and 2 mm (SRC 4, SRC 2) were marked with Dy and erbium (Er), respectively. The CC particles, 4 mm, 2 mm and 1 mm (CC 4, CC 2, CC 1) were marked with yttrium (Y), Yb or samarium (Sm), respectively. Cobalt ethylene-diaminetetra-acetate (CoEDTA) was used as the liquid marker. Although the turnover rate of CC 1 appeared to be 21 and 19 % faster than CC 2 and CC 4, respectively, corn particles ranging from 1 mm to 4 mm passed through the rumen at the same rate \((P > .05)\) regardless of the method of processing. Rare-earth markers were shown to be accurate estimators of ruminal turnover rates when samples were taken from the rumen.

\textit{\(^{91}\text{Yttrium}\).} Three radioactive markers \((^{91}\text{Y}, ^{51}\text{Cr-EDTA and}^{141}\text{Cerium, Ce})\) were tested in chicks to evaluate their use as reference substances for \textit{in vivo} intestinal absorption studies (Sklan et al., 1975). The \(^{51}\text{Cr-EDTA marker} \) is a \(\gamma\) emitter and is water-soluble, and
in the rumen is bound only minimally to solid particles (Downes and McDonald, 1964; Warner and Stacey, 1968). The $^{91}$Y marker is a $\beta$ emitter and is primarily bound to the solid particles in the intestinal tract of chickens (Hurwitz and Bar, 1972; Hurwitz et al., 1972, 1973), and in rats (Marcus and Lengermann, 1962). The $^{141}$Ce marker is mainly bound to solid particles in cattle (Miller et al., 1969), and either $\beta$ or $\gamma$ emissions are used for its determination. In trial 1, chicks were administered a single dose of $^{91}$Y, with $^{141}$Ce or $^{51}$Cr into the crop, and in trial 2, chicks were fed for 4 d a diet labeled with $^{91}$Y and $^{141}$Ce, or with $^{91}$Y and $^{51}$Cr (Sklan et al., 1975). The absorbability of the markers was evaluated by the recovery of the administered dose in the intestinal contents and feces, and the isotope blood levels after a single oral dose or after continuous isotope feeding. All three isotopes were equally well recovered (>95%) after a single oral dose.

When isotopes were fed continuously the ratios of $^{91}$Y to $^{51}$Cr showed differences throughout the intestinal tract, with a higher ratio in the crop and gizzard and a lower ratio in the duodenum. The $^{51}$Cr marker had a more rapid time of passage through the crop and gizzard and slower passage through the duodenum. As $^{91}$Y is tightly bound to solid particles, it is expected to travel slowly relative to fluids through the crop and gizzard. The slower passage of $^{51}$Cr through the duodenum reflects the passage of fluids through the spaces between villi compared to the solid particles passing directly through the lumen. Levels of $^{51}$Cr in the blood increased following both methods of dosing, although its recovery was not modified. The study showed that the $^{91}$Y and $^{141}$Ce markers were suitable reference substances for evaluating absorption in vivo, and the $^{51}$Cr marker was suitable for determining the absorption of substances in the liquid phase (Sklan et al., 1975).

**External markers**

External markers have most frequently been used to estimate FO, and in addition fecal kinetic estimates of PFM and TT can be determined (Merchen, 1988; Bertone et al., 1989; Holland et al., 1998). Marker estimates of FO can be verified by comparison with TC of feces (Krysl et al., 1985, 1988; Musimba et al., 1987).
**Chromium.** Chromic oxide (Cr\(_2\)O\(_3\)) has been widely used as a fecal marker in both radioactive and non-radioactive forms in studies of food utilization (Kotb and Luckey, 1972; Cuddeford and Hughes, 1990; Barbisan et al., 1993). The rate of passage in horses fed 2 hays (alfalfa or timothy) and a supplement (corn, oats or barley), was evaluated to establish the length of time necessary to obtain valid DMD estimates of diets (Vander Noot et al., 1967). After a 14 d dietary accommodation period, horses were dosed with Cr\(_2\)O\(_3\) (mimeograph paper covered in a Cr\(_2\)O\(_3\) and water paste) mixed in feed. Analysis of indicator paper showed that Cr\(_2\)O\(_3\) content varied considerably and the amount ingested by each horse could not be established accurately. Total collection of feces was made every 12 h for 6 d and approximately 100 % of Cr\(_2\)O\(_3\) ingested was recovered in feces within 96 h. The authors concluded that 96 h (4 d) was adequate for TC in digestibility trials of horses fed hay and supplement.

Fecal excretion rates of Cr\(_2\)O\(_3\) by horses were compared with standard TC measurements (Haenlein et al., 1966). The Cr\(_2\)O\(_3\) was administered via a balling gun twice daily for 10 d before 6 d of TC. Diurnal variation of fecal Cr\(_2\)O\(_3\) concentration was determined 3 times a day for 6 d. The recovery of Cr\(_2\)O\(_3\) averaged 98.4 % of administered dose. Diurnal variation in recovery of Cr\(_2\)O\(_3\) ranged from 59.8 to 134 % and the diurnal pattern was similar among 6 horses. Between day variations in Cr\(_2\)O\(_3\) recovery were as great as the values for diurnal variation. Estimates of Cr\(_2\)O\(_3\) in fecal grab samples collected over a 4 d period were similar to results of the 10 d TC procedure, provided that fecal recovery rate of Cr\(_2\)O\(_3\) was considered.

Assuming that representative fecal samples can be collected, a marker need not be associated with a particular digesta phase (particulate or fluid) to be an appropriate marker of total digesta passage (Titgemeyer, 1997). Chromic oxide seems to be inert in the gut, however, recoveries are often less than 100 %, and in a review of 90 nutrient digestion studies by Titgemeyer (1997) that used Cr\(_2\)O\(_3\) as the digestion marker, nine that reported fecal recovery of Cr, had an average of 94 % recovery.
Estimates of DMD and FO from fecal marker concentration were determined using fecal grab samples from beef cows dosed once or twice daily with Cr$_2$O$_3$ and Yb (Prigge et al., 1981). Fecal grab sample marker concentrations were compared to standard TC values. With single daily dosings, FO estimates from Cr$_2$O$_3$ were underestimated, whereas with twice-daily dosing, FO estimates closely approximated TC values. Relative Cr$_2$O$_3$ concentrations in grab samples collected at 4 h intervals indicated that twice-daily dosing reduced diurnal variation.

**Diurnal Variation.** Variation in marker concentration can be observed when intake of either marker or diet is sporadic. When marker concentrations in digesta samples vary diurnally, representative concentrations of markers can be achieved by sampling at various times throughout the day (Titgemeyer, 1997). Samples collected over time, to overcome problems with diurnal variation in marker concentration, ideally should be combined (Owens and Hanson, 1992). When marker flow is constant and total digesta flow exhibits variation, individual samples that each contain the same amount of marker should be combined. However, when total digesta flow is constant and the marker flow is affected by variability, equal amounts of each sample should be mixed. Because it is usually unclear whether the marker concentration is affected by variability in marker or digesta flow, or both, pooling of samples on the basis of equal digesta weight should be considered as an acceptable approach to decreasing diurnal variation (Titgemeyer, 1997).

Problems associated with the use of Cr$_2$O$_3$ include: fecal recoveries of Cr$_2$O$_3$ often deviate from 100 %, especially in grazing animals, large variations in fecal recovery of Cr$_2$O$_3$ among animals, fecal concentrations of Cr$_2$O$_3$ exhibiting large diurnal variation, lack of association with either the particulate or fluid phase of the ingesta, and large dose amounts required to attain accurate laboratory analysis. Problems of diurnal variation can be overcome if enough samples are collected throughout the day to provide an average sample in which the marker concentration is representative of that over the entire day. In addition, problems associated with Cr$_2$O$_3$ not associating specifically with either the particulate or fluid phase, can be diminished if representative samples are collected. Despite these problems, Cr$_2$O$_3$ is the most widely used of all digesta markers because it is
inexpensive relative to other external markers, readily incorporated into diets, and can be analyzed easily (Titgemeyer, 1997).

**Ytterbium.** Rare earth metals have been widely used in ruminant studies. Ytterbium has been researched extensively and has been used effectively as an external marker (Coleman, 1979; Teeter et al., 1979, 1984; Pond et al., 1986). It binds tenaciously with the fiber portion of a feed, remains with the particulate phase of the digesta (Ellis et al., 1980), is readily detected using atomic absorption spectrophotometry, and is relatively inexpensive.

Twelve rumen-cannulated lambs, fed alfalfa or prairie hay, were used to compare estimates of FO from a pulse-dose of ytterbium (Yb)-labeled forage and fecal collection bags (Krysl et al., 1985). Estimates of FO with Yb-labeled forage were not different from TC values for lambs fed either hay. Particulate passage from the rumen was faster in lambs fed alfalfa than in lambs fed prairie hay. A pulse dose of Yb-labeled hay was considered a reliable marker for estimating FO and passage rate estimates, although validation of techniques in free-grazing ruminants is needed.

Twenty rumen-cannulated lambs fed one of four diets, were used to compare estimates of FO from a pulse-dose of ytterbium (Yb)-labeled forage to standard TC procedures (Krysl et al., 1988). The four diets were, prairie hay (PH), lucerne hay (LH), 50% prairie hay:50% sorghum grain (PS), and 50% lucerne hay:50% sorghum grain (LS). The Yb-labeled forage was pulse-dosed via ruminal cannulae, and a fecal Yb excretion curve was fitted to a one-compartment model for estimation of FO. The FO for lambs fed PH did not differ from marker estimates but was overestimated by 15 to 20% by Yb-labeled forage. The FO for lambs fed LH was similar to the marker estimate, and for lambs fed the PS diet, FO did not differ from marker estimates although Yb-labeled forage values were 16% lower. No differences were observed in actual and estimated FO for lambs fed the LS diet.

Experiments were conducted with sheep in pens, to determine the distribution of Yb recovery over time for diets with high and low DMD, and in field conditions, to estimate
supplement intake using Yb as a marker (Curtis et al., 1994). In Exp.1, 18 sheep were placed in metabolism crates and fed wheaten chaff plus a lupin seed supplement in the following amounts (g): 700 + 0, 525 + 175 (3:1), or 175 + 525 (1:3). Diets were fed for three weeks, followed by 7 d TC, and Yb was administered via stomach tube 24 h before the first TC. In Exp.2, 100 sheep in large grazing paddocks, with 50 sheep fitted with fecal collection bags, were fed the supplement from a feeder where supplement (600 g/sheep) was dispensed daily. The Yb was added to the supplement over a 24 h period, followed by 5 d TC. For Exp.1, mean retention time (MRT) of Yb in the digestive tract for each ration was estimated using a Gamma 2, age-dependent, age-independent model, fitted for each sheep (Quiroz et al., 1988).

In Exp.1, FO was higher for sheep on wheaten chaff only, than for sheep fed either wheat:lupin (3:1) or wheat:lupin (1:3) diets. The FO for sheep fed wheat:lupin (3:1) was higher than for sheep fed wheat:lupin (1:3). The apparent DMD estimated from TC were lowest in the wheaten chaff only diet and highest in the wheat:lupin (1:3) diet. Using the DMD of the wheaten chaff only diet, the lupin seed supplement DMD for the mixed rations was calculated. The MRT of Yb in the GIT for the three rations were similar, and the distribution of recovery was independent of the level of lupin seed in the ration. This is critical to the use of this marker in field conditions where lupin seed intake by individuals is likely to vary greatly. In Exp.2, FO were variable both between sheep and between days for individual sheep. The estimated intake of lupin seed from recovery of Yb over 5 d following feeding, was 9.5 % lower than dispensed intake. When unrecovered Yb was taken into account, the difference between lupin seed intake estimated from Yb recovered and Yb dispensed was 5.2 %, and it was concluded that Yb may be used as a marker for estimating intake of lupin seed by sheep grazing in a group (Curtis et al., 1994).

Ten lambs were fitted with fecal collection bags, confined in metabolism crates and orally administered either a pulse dose or a once daily dose of Yb for 7 d. The accuracy and precision of different methods of administering Yb as a marker to estimate FO were evaluated (Hatfield et al., 1989). Both methods overestimated FO (7.3 % for the once daily dose and 11.2 % for the pulse dose method), however, the once daily dose was more precise
In a subsequent experiment, steers grazing dormant brome grass pasture were used to compare estimates of FO by pulse dosing and once daily dosing of Yb via rumen cannula. Although both methods underestimated FO (4.0 % for the once daily and 11.5 % for the pulse dose), once daily dosing was more precise (SE = 2.36 %) than pulse dosing (SE = 3.64 %).

Three balance experiments were conducted to determine the DMI and apparent DMD of tall fescue and orchardgrass/clover pastures in horses, in different seasons of the year (Moffitt, 1987). A double marker procedure was used with indigestible neutral detergent fiber (INDF) to estimate DMD, and Yb to estimate FO and subsequent DMI. Ten horses were divided into two groups and assigned to either a tall fescue pasture, or an orchardgrass/clover pasture, and were allowed a 10 d adjustment period. Each horse was administered the Yb via a pulse-dose of Yb-stained forage mixed with ground corn, and fecal grab samples were collected every 6 h for 3 d. Orchardgrass/clover DMD was not different between winter and spring, but was higher (P < .05) in the summer. Tall fescue DMD was higher in the winter. The DMI and FO were not different among horses within trials and forages. Orchardgrass/clover DMI was lowest (P < .05) in the winter, and tall fescue DMI was highest (P < .05) in the summer.

Three balance experiments were conducted to determine the DMI, FO and apparent DMD of tall fescue pastures in horses, in different seasons of the year (Meacham, 1987). A double marker procedure was used with indigestible neutral detergent fiber (INDF) to estimate DMD, and Yb to estimate FO and subsequent DMI. After a 14 d adjustment period, five horses were administered a pulse-dose of Yb-stained forage, and fecal grab samples were collected every 6 h for 3 d. Estimates of FO were calculated from fecal Yb concentration. Estimated DMI and fecal DM were higher (P < .05) in the winter, and estimated DMD were lower (P < .05) for the spring forage. In these studies (Moffitt, 1987 and Meacham, 1987), estimates of DMD using INDF, and FO estimates using Yb were not compared to total collection of feces, therefore validation studies need to be conducted to justify the use of these markers in future studies.
**Fecal Kinetics**

In tracer kinetics a system is the part of the universe of interest (the body, part of the body), and markers or tracers provide information on the amount, distribution and movements of the tracee in a system. The study of a living system in complete detail is essentially impossible, and experimental observations describing only a portion of the system are subject to limited precision and resolution. A system is analyzed by simplifications or models based on the data describing the time course of distribution of tracers or markers into, through and out of the system. Kinetics describe the temporal and spatial interrelationships of the tracer (or marker) and the system. Model building increases the uniqueness of a model, and reveals weaknesses in data which should lead to further experiments, and in turn lead to improved models (Brownell et al., 1967; Kronfeld and Ramberg, 1981).

A one-compartment model applies calculations more widely applied to tracer-dilution than to marker experiments (Brownell et al., 1968; Merchen, 1988). When a known dose of a tracer or marker is administered into a system, followed by a series of sample collections, the temporal response of the marker concentration can be observed (Brownell et al., 1968). In non-compartmental analysis the flow rate of marked material can be calculated from the magnitude of the temporal response. This magnitude can be calculated as the area subtended by the time-concentration curve following a pulse dose of tracer, or as the asymptotic concentration during a continuous infusion of tracer (Meier and Zierler, 1954). In compartmental analysis, additional information on the structure of the system is obtained from the shape of the temporal response by analyzing the whole curve and determining the rate constant, which can be used to calculate the TT and mixing mass or compartment size (Brownell et al., 1968; Kronfeld and Ramberg, 1981).

The use of digesta flow markers to study the quantitative kinetics of liquid or particulate matter within the GIT of ruminants is well established (Ellis et al., 1979; Faichney, 1986; Grovum and Williams, 1973; Moore et al., 1992). Ruminal volume at a specific time can be calculated through extrapolating to concentration of a marker at dosing time. Marker estimates of volume can be imprecise due to delays in mixing and dispersion.
of the marker at the site of dosing, unrepresentative sampling and diurnal variation (Owens and Hanson, 1992). When the sampling of feces is frequent, various kinetic models of flow can be fitted to fecal marker concentration data to estimate pool sizes and rate constants. Fecal appearance of the marker is delayed because of the time for transit through the GIT, so a time delay function must be included in the model. A variable number of pools and mixing delays can be incorporated into these models. Models that incorporate time delays together with two or more pools may improve data fit, however, statistical interpretation of multipool systems is complex (Oltjen et al., 1986; France et al., 1988; Owens and Hanson, 1992).

In studies of two-compartment systems in sheep, cattle and horses, marker methods for estimating transit time and mean retention time (MRT) have been combined with kinetic estimates of rate constants, and/or an age-dependent (time dependent) rate function, or delay that substitutes for one rate constant (Blaxter et al., 1956; Grovum and Williams, 1973; Pond et al., 1988; Bertone et al., 1989). Estimates of passage rate and retention times were calculated from a two-compartment model, where the natural log of fecal marker concentration was plotted against time, and ruminal passage rate was calculated as the slope of the linear, descending portion of the line (Grovum and Williams, 1973).

Digesta kinetic estimates derived from Cr-mordanted hay or pellets were compared to estimates derived from rare-earth markers (Yb, Dy, or Er) applied individually to samples (Moore et al., 1992). Rams were fitted with fecal collection bags and dosed with marked feed in gelatin capsules. Digesta kinetics were obtained both by nonlinear analysis with two age independent rates (G1G1), or with increasing orders of gamma age dependency (G2G1 to G4G1), and by linear regression of natural log (LN) transformed fecal marker concentrations. These models estimated passage from two compartments (fast and slow, with the slow compartment representing passage out of the rumen) and included a time delay.

For both diets, ruminal outflow rate, ruminal MRT, and total tract MRT were different for the Cr-mordanted vs rare earth marked feeds (Moore et al., 1992). The Cr-
mordanted particles remained longer in the rumen and total tract, suggesting that the rare earth markers may have partially dissociated from the marked particles and entered the more rapidly flowing liquid portion of digesta. Despite the marker used, relative comparisons of the two diets did not change and the pelleted diet had faster passage and shorter retention times than the hay diet. Total retention time was similar ($P > .21$) for the LN, G3G1, and G4G1 models. Nonlinear models overestimated, and LN underestimated the fecal DM output by 9% ($SEM = 4.7$) for the hay diet. All nonlinear models provided fecal DM output estimates for the pelleted diet that were within 5% of actual fecal DM output, but the LN model underestimated it by 18% ($SEM = 3.3$). The three rare earth markers gave identical results for digesta kinetic estimates, illustrating their use for simultaneous study of more than one ingredient or particle (Moore et al., 1992).

A study was conducted to evaluate alterations in the kinetics of particulate matter flow through the gastro-intestinal tract of horses after extensive large colon resection, compared with kinetics before surgery and with that of sham-operated control horses (Bertone et al., 1989). Particulate flow trials were conducted on 9 horses 1 to 2 wk before surgery, 3 wk, 3 mo, and 6 mo after surgery. Horses were fed a limited intake of alfalfa pellets for 10 d, with fecal samples collected three times a day during the last 5 d of each trial. Ytterbium chloride was administered to each horse in the morning feed on d 5. The natural log slope of the descending portion on the Yb excretion curve ($k_1$) was calculated and represented the fractional rate of particulate passage of the slow moving pool. The ascending portion of the Yb excretion curve ($k_2$), together with $k_1$ were multiplied by 100 and percent passage per hour was determined. The linear equation of the natural log slope of the descending portion of the Yb excretion curve was extrapolated back to the time of first appearance of marker (transit time) to estimate the capacity (kg of dried ingesta) of the mixing pools represented by $k_1$. The inverse of the fractional rates of particulate passage ($1/k_1$ and $1/k_2$) was calculated and represented the TT for the slow and fast mixing pools, respectively.

Colon resected horses had shorter transit, peak and mean overall retention times, compared with preoperative values and with values for sham-operated horses. A slower
emptying pool \((k_1)\) and a faster emptying pool \((k_2)\) were identified. The first pool \((k_1)\) was not altered by colon resection and approximated the capacity of the cecum, which expanded by 6 mo in the resected horses. The rate of passage from the second pool \((k_2)\) increased initially after colon resection (3 wk and 3 mo), but returned to preoperative values by 6 mo. This pool \((k_2)\) was affected by colon resection and was interpreted as being influenced by a portion of the colon (Bertone et al., 1989).

A one-compartment model using chromic oxide can be used to represent fecal kinetics in horses (Holland et al., 1998). The main assumption in the model was that chromic oxide particles were mixing thoroughly in the prefecal mass (PFM) of suspended and dissolved material, and that chromic oxide was not mixing in the delay component between the mouth and the PFM. Four horses were fed hay, and another four were fed hay and concentrate. Balance-marker experiments were conducted for 10 d, and a dose of chromic oxide mixed in chopped hay and molasses was administered from a nose-bag daily for 10 d. Fecal chromium data were fitted to a single exponential, with one rate constant, rising to an asymptote. A delay was introduced between the pulse oral dose and the entry of the marker into the prefecal pool. The rate constants yielded turnover times of 33 and 18 h, and PFM of 4.6 and 2.9 kg of DM for hay or hay and concentrate groups, respectively. Marker estimates were correlated with total collection estimates of FO. Recoveries of Cr were 108 and 115 % dose for the hay and hay plus concentrate diets, respectively. The authors suggested that if Cr doses could be administered more frequently than daily, the model would generate more accurate and precise estimates of FO (Holland et al., 1998).