Fecal Kinetics and Digestibilities of Hays and Supplements Estimated by Marker Methods in the Horse

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(ABSTRACT)

Marker methods are being developed to measure fecal output, digestibility and intake of pasture by grazing horses. A marker model of fecal kinetics consists of removal of feces at a constant rate from a single compartment, the prefecal mass. Following the preliminary work of Holland et al., (1998), three improvements in experimental design were tested. First, the rate constant was determined from the post administration curve of fecal concentration versus time, as well as the curve during marker administration. Using more points should better determine the rate constant. Second, the chromium (Cr) marker was administered three times a day, instead of once, to reduce diurnal variation in fecal marker concentration. Third, yttrium (Y) and ytterbium (Yb) were tested as internal markers, for the estimation of digestibility of hay and supplements, respectively.

Eight horses were fed Diet 1 (orchardgrass/alfalfa mixed, OG) or Diet 2 (tall fescue/alfalfa mixed, TF) in Exp.1, and Diet 3 (OG plus fat-and-fiber supplement, OGFF) or Diet 4 (OG plus sugar-and-starch supplement, OGSS) in Exp.2. Balance-marker experiments were conducted for 17 and 20 d, with 7 and 10 d of dietary accommodation in Exp.1 and 2, respectively. Chromic oxide and Yb were administered orally and fecal samples were collected every 8 h for 8 d. Dry matter, Cr, Yb and Y were measured in feeds and feces.

In balance experiments, estimates of DMD (D_E) using Y, were determined precisely (SE 1 to 3 %) for hay and hay and supplement diets. Linear relationships, correlations and calibration curves were determined, validating Y as a marker.

Mean daily fecal Cr data (C_t) at time t (days) including a delay (d) were fitted to a single exponential, with one rate constant (k), rising to an asymptote (C_a):

$$C_t = C_a - C_a \cdot e^{-k\,(t-d)}$$

Diets 1 and 2 had two sets of C_t data, total collection (a) and fecal grab data (b), and each set was used in model development. Diets 3 and 4 had two sets of C_t data (both using fecal grab data), Cr marker dilution (3Cr and 4Cr) and Yb marker dilution (3Yb and 4Yb).

For pooled data, delays of 3 to 6 h (Diets 1a, 1b, 2a and 2b) and delays of 5 to 7 h (Diets 3Cr, 4Cr, 3Yb and 4Yb) gave best fits (highest estimates of R^2). The delays introduced to the Cr model for both 3Cr and 4Cr diets did not correspond to the preliminary study (Holland et al., 1998), where a 2 h delay gave the best fit in the model for horses fed hay and supplement. The present estimates may more realistically relate to mouth-to-cecum transport times, because the marker was administered three times a day instead of once, and the initial part of the tracer curve was more precisely defined.

The results showed that fecal Cr kinetics could be calibrated precisely (SE 1 to 3 %) to predict fecal DM output of horses fed Diets 1b, 2b, 3a but not 4a. Similarly, fecal Yb kinetics could be calibrated to predict fecal DM ouput of horses fed Diet 3b but not 4b.

The rate constants yielded turnover times (TT) that were longer with hay and supplement diets, than with hay alone, and which contrast with previous findings in the horse. However, the longer TT were similar to slower rates of marker excretion in sheep fed concentrates instead of all-roughage diets, suggesting that the lower fiber content retarded the rate of propulsion of digesta through the digestive tract. For two of the eight models of fecal kinetics, the rate constants of the post-administration curve were not well determined by the data, and rate constants from the administration curve were used. In future experiments, more frequent fecal sample collection during the post-administration period may improve rate constant determination.

Improvements in diurnal variation of fecal marker concentration were obtained by dosing three times a day. But discrepancies between Cr and Yb concentration means of diurnal samples and combined samples showed incomplete mixing, the major source of tracer error. Therefore more frequent marker administration and fecal samples should be tested in future experiments to achieve more thorough mixing in the prefecal mass for modeling fecal kinetics, and in the small intestine for estimating digestibility.

(Key Words: markers, fecal output, turnover, digestibility, horse)

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Introduction

Pastures provide the main habitat and nutrition of most horses, and are especially important for brood mares, foals and yearlings. In Virginia, about 40 % of 225,400 commercially active horses are kept on pasture full-time, another 40 % part-time, and 20 % are stall confined (Virginia Horse Industry Profile, 1995, 1996). Approximately half of the nutrition of stall confined horses is supplied by conserved pasture or hay. The environmental impact of horses on plants, soils and streams needs to be considered, in particular the excretion of nitrogen and phosphorus which contaminates soils and water. The *mission* of the Middleburg Agricultural Research and Extension Center is to develop an ideal pasture system that will not only improve the care and nutrition of horses, but also protect and enhance the land (Kronfeld, 1998). Improvements in methods of measuring pasture intake, pasture and supplement digestibility, and fecal output are essential for research on pasture-animal systems, and in the development of an optimal nutritional supplement for pastures.

Current methods for estimating the daily intake of energy and nutrients of grazing horses are imprecise because pasture consumption has not been measured directly. In several grazing species, pasture intake has been measured indirectly by indigestible substances or *markers* (Kotb and Luckey, 1972; Owens and Hanson, 1992; Mayes et al., 1995), applying various dilution concepts and calculations (Blaxter et al., 1956; Kronfeld and Ramberg, 1981). The first markers used in simple-stomached animals were indigestible colored substances such as ferric oxide or carmine (McDonald et al., 1981). These markers were added to the first and last meals of the experimental period and feces could be identified with corresponding meals. The beginning and the end of fecal collection were delayed until the dye appeared in and disappeared from the excreta. In effect, the markers were used to relate periods of feed intake to corresponding periods of fecal output. Since this first use of dyes as markers, dyes and other substances have been used as indicators or tracers to estimate digestibility and rates of passage of ingesta through the digestive tract (Blaxter et al., 1956). Numerous internal and external markers have been developed and

tested in ruminants and other herbivores (Sutton et al., 1977; Krysl et al., 1988; Owens and Hanson, 1992).

Markers and tracers are labeled molecules, substances or particles used to measure certain properties of a system (Brownell et al., 1968). The amount, distribution and movements of the tracee in a system is derived in the form of models based on data describing the time course of distribution of tracers or markers into, through and out of the system (Brownell et al., 1956; Kronfeld and Ramberg, 1981). The term *kinetics* is used to represent a mathematical description of the temporal and spatial interrelationships of the tracer (or marker) and the system. Approximations of tracer kinetics have been used more commonly than rigorous calculations in applications of markers to digestive physiology in livestock (Merchen, 1988; Galyean, 1993; Titgemeyer, 1997).

In the nutritional evaluation of grazing animals, individual measurements of dry matter intake (DMI), dry matter digestibility (DMD), and fecal output (FO) are necessary. Estimates of DMD can be calculated from the known DMI and FO by total collection, or be calculated from the concentration of marker in the feed and feces. Estimates of FO can be calculated from the dose of an external marker and the asymptotic fecal marker concentration. Concurrent estimates of FO and DMD allow calculation of pasture DMI.

In this study, a marker model of fecal kinetics using Cr or Yb is being developed for grazing horses, following the preliminary work of Holland et al., (1998). Improvements in experimental design will be tested in experiments on stall-fed horses in the context of digestion balance trials. Improvements will be to continue fecal sample collection for 6d after the last day of marker administration, allowing the rate constant to be determined both by the administration and the post-administration curve of the one-compartment model of fecal kinetics. Also, markers will be administered three times a day instead of once daily to reduce diurnal variation of fecal marker concentration. In the preliminary study of Holland et al., (1998) acid detergent lignin and acid insoluble ash internal markers gave poor estimates of digestibility. On this account, the present research will evaluate yttrium (Y) and Yb markers as internal markers for estimating DMD of hay and hay plus supplement diets.

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 (Kotb 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).

An important focus in equine nutrition research is to develop methods that accurately and precisely estimate DMI of grazing horses. A double marker method with AIA to estimate DMD, and Cr_20_3 to estimate FO, was used to determine the DMI of grazing mares (Martin et al., 1989). Mares were divided into pregnant or lactating groups and were either supplemented or non-supplemented. Marker estimated pasture DMI was higher ($P < .05$) for non-supplemented mares (9.0 kg) than supplemented mares (7.6 kg). The pasture DMI was lower ($P < .01$) for pregnant mares (7.0 kg) than for lactating mares (9.6 kg), indicating an increased nutritional demand during lactation.

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. The highest concentration of alkanes (> 90%) are odd chain carbon lengths between C21 and C37 (Tulloch, 1976). Alkane fecal recoveries in ruminants increased with increasing chain length (Mayes and Lamb, 1984), and ruminal digestion was thought to contribute to higher losses of shorter chain lengths relative to longer chain alkanes. More recent research has suggested that

alkane recoveries may not be affected by increasing carbon chain lengths in non-ruminants (Dove and Mayes, 1996; O'Keefe and McMeniman, 1998). Companion observations on alkanes in the present experiments confirm similar recoveries of alkanes for C27 to C 35 in horses (Ordakowski, 1998). In contrast to ruminant studies, fecal recoveries of odd-chain alkanes did not increase with increasing carbon chain length in preliminary indoor studies with horses, ponies and pigs (Mayes et al., 1995). In horses fed seven diets each containing different proportions of oaten chaff, alfalfa chaff, horse pellets, and cottonseed meal, recovery of n-alkanes of plant origins were higher than those in ruminants. In addition, n-alkane recovery did not increase with increasing carbon chain length (O'Keefe and McMeniman, 1998).

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}Lu. However, passage rate of particles administered at the beginning of the meal (^{160}Tb) was 42 % higher than for particles at the end of the meal (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).

^{89}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.

^{91}Yttrium. Three radioactive markers (^{91}Y, ^{51}Cr-EDTA and ^{141}Cerium, Ce) were tested in chicks to evaluate their use as reference substances for *in vivo* intestinal absorption studies (Sklan et al., 1975). The ^{51}Cr-EDTA marker is a γ 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 β 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 β or γ 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 ($Cr_2 0_3$) 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 $Cr_2 0_3$ (mimeograph paper covered in a $Cr_2 0_3$ and water paste) mixed in feed. Analysis of indicator paper showed that $Cr_2 0_3$ 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 $Cr_2 0_3$ 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 $Cr_2 0_3$ by horses were compared with standard TC measurements (Haenlein et al., 1966). The $Cr_2 0_3$ was administered via a balling gun twice daily for 10 d before 6 d of TC. Diurnal variation of fecal $Cr_2 0_3$ concentration was determined 3 times a day for 6 d. The recovery of $Cr_2 0_3$ averaged 98.4 % of administered dose. Diurnal variation in recovery of $Cr_2 0_3$ ranged from 59.8 to 134 % and the diurnal pattern was similar among 6 horses. Between day variations in $Cr_2 0_3$ recovery were as great as the values for diurnal variation. Estimates of $Cr_2 0_3$ 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 0_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_20_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_20_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_20_3 were underestimated, whereas with twice-daily dosing, FO estimates closely approximated TC values. Relative Cr_20_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_20_3 include: fecal recoveries of Cr_20_3 often deviate from 100 %, especially in grazing animals, large variations in fecal recovery of Cr_20_3 among animals, fecal concentrations of Cr_20_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_20_3 not associating specifically with either the particulate or fluid phase, can be diminished if representative samples are collected. Despite these problems, Cr_20_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 (SE = .78 %) than the pulse dose (SE = 2.17 %). 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).

Objectives

The general objective of this study was to develop marker methods for determining pasture consumption, digestibility and fecal kinetics in the horse. More accurate and precise measurements of these variables are needed for experiments on grazing animals. In addition, this research aims to improve and test the Cr model of fecal kinetics following the preliminary work of Holland et al., (1998).

The improvements will be to:

a) Determine the rate constant using more points
b) Reduce diurnal variation by administering the markers three times a day
c) Estimate dry matter digestibility of four diets using a novel internal marker.

Further specific aims:

1. Determination of fecal recovery of yttrium, chromium and ytterbium, in hay (orchardgrass/alfalfa vs tall fescue/alfalfa), and hay (orchardgrass/alfalfa)-plus-supplement (Fat-and-Fiber vs Sugar-and-Starch) diets offered to horses in stalls.

2. Evaluation of endogenous yttrium as an internal marker for the estimation of hay and hay-plus-supplement digestibility.

3. Evaluation of exogenous ytterbium distributed evenly throughout a supplement representing an 'internal marker' for the estimation of supplement digestibility.

4. The development of a ytterbium-model of fecal kinetics.

5. Comparison of the chromium-model and ytterbium-model of fecal kinetics.

6. Comparison of digestibility estimates and fecal kinetics of two hay diets and two hay-plus-supplement diets.

Materials and Methods

A marker model was tested in preliminary trials on stall-fed horses in the context of two digestion balance experiments consisting of two periods each. In Exp.1 horses were offered hay, and in Exp.2, hay and supplement.

Animals

Eight clinically healthy Thoroughbred geldings were housed in 4 x 4 m^2 individual box stalls and hand-walked daily for 15 min to provide limited exercise. Horses were between 6 and 12 years old and weighed 551 ± 63 kg (mean ± SE) and 548 ± 62 kg for Exp. 1 and 2, respectively. The protocol was approved by the Institutional Animal Care and Use Committee.

Dietary Treatments

Experiment 1. Horses were randomly assigned to two groups of four, each fed one of two diets in period 1, the other in period 2 (Appendix table 1). Diet 1 (OG) consisted of orchardgrass (*Dactylis glomerata*) and alfalfa (*Medicago sativa*) mixed hay, and Diet 2 (TF) tall fescue (*Festuca arundinacea*) and alfalfa mixed hay (Appendix table 2). Hays were second cuttings harvested in late June 1997 from the Virginia Tech Middleburg Agricultural Research and Extension Center. Each horse was initially fed at 2% of its body weight (BW), and its ration adjusted weekly to maintain BW.

Experiment 2. Horses were randomly assigned to two groups of four, each fed one of two diets in period 1, the other in period 2 (Appendix table 3). Diet 3 (OGFF) consisted of orchardgrass/alfalfa hay plus a fat-and-fiber (FF) supplement, and Diet 4 (OGSS) consisted of orchardgrass/alfalfa hay and a starch-and-sugar (SS) supplement (Appendix table 4). Horses were initially fed hay to meet 50% and supplement to meet 50% of the daily energy requirement for horses at stall maintenance (NRC, 1989). Hay intake was doubled on d 3 of the dietary accommodation period to eliminate coprophagy. Hay was a second cutting harvested in late June 1997 from the Virginia Tech Middleburg Agricultural

Research and Extension Center. The FF supplement included corn oil and four fiber sources (Appendix table 5), and the SS supplement was a commercial supplement (Omelene 200, Purina Mills, Inc., St. Louis, MO) high in starch and sugar.

Experimental Protocol

Experiment 1. The digestion balance experiment consisted of two periods of 17 d each. It was conducted in February 1997 (period 1) and March 1997 (period 2), with two weeks of turnout between periods. Two weeks prior to the start of period 1, and during the two weeks between periods 1 and 2, all horses had access to their respective experimental diets while on pasture. All horses were weighed and placed in stalls on d 1, and in each period were accustomed to the stalls and accommodated to the diets from d 1 to d 7. Horses were re-weighed on d 7 and d 14 so that dietary adjustments could be made throughout periods. Hay was weighed and offered in a net three times a day (0700, 1500 and 2300), and orts were collected and weighed each morning. Hay samples were collected every second day from d 1 to d 11. Water was available *ad libitum*, and its intake was recorded daily.

Base-line fecal grab samples were collected once a day on d 1 to d 3. On the following eight days (d 4 to d 11) horses were administered a granola bar (Cr_{GB}) marker three times a day (0700, 1500 and 2300), which contained 19.30 g Cr_20_3 (Chromium Oxide Sesqui; Fisher Scientific, Fair Lawn, NJ). Composition of Cr_{GB} are shown in Appendix table 6. Two Cr_{GB} samples from each batch of 80 bars made were retained for Cr analysis. From d 4 to d 17 fecal grab samples (about 650 g wet weight) were collected three times a day (0700, 1500 and 2300) and were combined daily for each horse. Also, all feces passed were collected every 1.5 h from d 4 to d 17, and each days output was weighed and mixed thoroughly. A representative daily sample (10% of wet weight) of the total collection from d 8 to d 11 was mixed thoroughly with a known volume of water to facilitate mixing. On d 11, three additional fecal grab samples were collected from each horse and used to evaluate diurnal variation of fecal Cr concentration.

Experiment 2. The digestion balance experiment consisted of two periods of 20 d each. It was conducted in mid-April to mid-May 1997 (period 3) and July 1997 (period 4).

Two weeks prior to the start of each period, all horses were offered their respective experimental diets while on pasture. All horses were weighed and placed in stalls on d 1, and were accustomed to the stalls and accommodated to the diets from d 1 to d 10. Horses were re-weighed on d 7, d 14 and d 20. Hay was weighed and offered in a net five times a day (0700, 1100, 1500, 1900 and 2300), and orts were collected and weighed each morning. Supplement was weighed and offered in a secured feed tub three times a day (0700, 1500 and 2300) and was completely consumed. Hay and supplement samples were collected every second day from d 4 to d 18. Water was available *ad libitum*, and its intake was recorded daily.

Base-line fecal grab samples were collected once a day from d 4 to d 6. On the following eight days (d 7 to d 14) horses were administered Cr_{GB} markers at 0700, 1500 and 2300. In addition, each horse was administered 1.5 g of $YbCl_3 \cdot 6H_2O$ (Ytterbium (III) Chloride hexa-hydrate, Aldrich Chemical Company, Inc. Milwaukee, WI) at 0700, 1500 and 2300, on d 7 to d 14. The Yb was dissolved in 200 ml of warm (approximately 80°C) water and mixed thoroughly into the supplement. From d 7 to d 20 fecal grab samples were collected three times a day (0700, 1500 and 2300) and were combined daily for each horse. Also, all passed feces were collected every 1.5 h from d 7 to d 20, and each day's output was weighed and mixed thoroughly. On d 14, three additional fecal grab samples were collected from each horse and used to evaluate the diurnal variation of fecal Cr concentration. Two Cr_{GB} samples from each batch of 80 bars made were retained for Cr analysis.

Sample Analyses

Hay, supplement, Cr_{GB} and fecal samples were individually weighed and dried in a 100°C forced air oven then ground through a .5mm screen Cyclone Mill (Model 3010, UDY Corp., Fort Collins, CO). Composite samples of ground hay, supplement (d 1 to d 11 in Exp.1, and d 4 to d 18 in Exp.2) and feces combined daily for each horse (d 8 to d 11 in Exp.1, and d 11 to d 14 in Exp.2) were mixed thoroughly, and two subsamples were submitted (Dairy One, Ithaca, NY) for duplicate analysis of dry matter, crude protein, crude fat, ash, NDF, ADF, NSC, Ca, P, Mg, Na, K, Fe, Zn, Cu, Mn, Mb and S (Robertson and Van Soest, 1977; AOAC, 1990).

Composite samples of ground hay and supplement (d 1 to d 11 in Exp.1, and d 4 to d 18 in Exp.2) were mixed thoroughly, and two subsamples were analyzed in duplicate for chromium (Cr) concentration in Exp.1 and for Cr and ytterbium (Yb) concentration in Exp. 2 (Equine Nutrition Laboratory, Virginia Tech, Blacksburg, VA). Individual horse fecal grab samples combined for each day (d 1 to d 17 in Exp.1, and d 4 to d 20 in Exp.2) were anlayzed for Cr and Yb concentration. Individual horse fecal grab samples collected for diurnal variation analysis on d 11 and d 14 of Exp.1 and 2, respectively, and the representative samples from total collection (Exp. 1), were also analyzed for Cr and Yb concentration. From each experiment, 16 Cr_{GB} samples were analyzed in duplicate for Cr concentration (Equine Nutrition Laboratory, Virginia Tech, Blacksburg, VA).

Chromium Analysis. Hay, supplement and fecal samples (.500 g) and Cr_{GB} samples (.1 g) were accurately weighed and placed in 50 ml digestion tubes. Concentrated nitric acid (4 ml) was added to each tube and samples were digested for 24 to 48 h. Perchloric acid (70%) was added (2 ml) to each tube and samples were placed on a digestion block for approximately 3 h (Sandel, 1959). Initially the heat was low to burn the nitric acid, then heat was gradually increased to fully digest the samples. Samples were cooled in a fume hood overnight, then resuspended to 35 ml with deionized water, and vortexed. An aliquot (14 ml) was placed in polypropylene tubes, sealed and stored pending Cr analysis. Dilutions, either 1:25 or 1:50 were made. The original dilution of all samples was .500 g in 35 ml which is a 1:70 dilution, so a 1:70 dilution x 1:25 dilution = 1,750 total dilution, and a 1:70 dilution x 1:50 dilution = 3,500 total dilution. Samples were analyzed for Cr by atomic absorption spectrophotometry (AAS, Model Zeeman 5100, Perkin Elmer) at 357.9 nm in an air-acetylene flame. Standards prepared by dilutions of a 100 ppm stock standard solution were used for calibration.

Ytterbium Analysis. Hay, supplement and fecal samples (.500 g) were digested as described for Cr analysis. The original dilution of the samples was .500 g in 35 ml that is a 1:70 dilution and no further dilutions were made. Samples had .1 % KCl added to prevent ionization interference. Samples were analyzed for Yb by AAS at 398.8 nm in a nitrous

oxide-acetylene flame. Standards prepared by dilutions of a 100 ppm stock standard solution were used for calibration.

Composite samples of ground hay, supplement (d 1 to d 11 in Exp.1, and d 4 to d 18 in Exp.2), and feces combined daily for each horse (d 8 to d 11 in Exp.1, and d 11 to d 14 in Exp.2) were mixed thoroughly, and two subsamples were submitted (Soils Testing Laboratory, University of Israel, Rehovot, Israel) for duplicate analysis of yttrium (Y) concentration.

Yttrium Analysis. Hay, supplement and fecal samples (250 g) were accurately weighed and then digested in 5 ml of concentrated nitric acid for 10 min (Sandel, 1959). Samples were heated for 10 min in microwave at 580 W and resuspended up to 25 ml with deionized water. Samples were analyzed for Y by inductively coupled plasma (ICP) spectrophotometry (Spectroflame, Spectra, Germany), at 371.03 nm in an argon-plasma flame. The standards used were 1000 ppm (Merck, Sharp and Dohme Research Laboratories, Chemical division, Rahway, NJ).

Digestibilities of Hays and Supplements - Calculations

Experiment 1. Total collection DMD (D_{TC}, %) of each diet was calculated using mean diet intake (I, kg/d DM) and mean fecal output (FO_{TC}, kg/d DM) from d 8 to d 11:

$$D_{TC} = (1 - (FO_{TC} / I)) \bullet 100 \qquad [1]$$

Fecal recovery of yttrium (R_Y, %) for each horse offered each diet was calculated using mean I, FO_{TC}, and Y concentration in diets (C_I, mg/kg DM) and feces (C_{FO}, mg/kg DM):

$$R_Y = ((FO_{TC} \bullet C_{FO}) / (I \bullet C_I)) \bullet 100 \qquad [2]$$

Estimated DMD (D_E, %) of the diets for each horse were calculated using C_I and C_{FO} of Y:

$$D_E = (1 - (C_I / C_{FO})) \bullet 100 \quad [3]$$

Experiment 2. The D_{TC} of the diets were calculated for each horse using mean hay intake (I_H, kg DM/d), mean supplement intake (I_S, kg DM/d), and FO_{TC} from d 11 to d 14:

$$D_{TC} = (1 - (FO_{TC} / (I_H + I_S))) \bullet 100 \quad [4]$$

Daily Yb dosage for each horse was calculated from the 1500 mg of $YbCl_3 \bullet 6H_2 0$ administered to each horse, and was mixed thoroughly in the supplement to represent an internal marker:

$$\text{Yb concentration in 1500 mg of } YbCl_3 \bullet 6H_2 0 = 670 \text{ mg} \quad [5]$$
$$\text{Dose} = 670 \text{ mg /d}$$

Fecal recovery of yttrium (R_Y, %) and ytterbium (R_{YB1}, %) for each horse offered each diet were calculated using I_H, I_S, FO_{TC}, and Cr, Y or Yb concentration in hay (C_H, mg/kg DM), supplement (C_S, mg/kg DM), and C_{FO} :

$$R_Y = ((FO_{TC} \bullet C_{FO}) / ((I_H \bullet C_H) + (I_S \bullet C_S))) \bullet 100 \quad [6]$$

$$R_{YB1} = ((FO_{TC} \bullet C_{FO}) / \text{Yb dose}) \bullet 100 \quad [7]$$

Estimated DMD (D_E, %) of diets 3 and 4 for each horse, with the Y internal marker, were calculated using I_H, I_S, C_H, C_S, and C_{FO} of Y:

$$D_E = (1 - (((I_H \bullet C_H) + (I_S \bullet C_S)) / (I_H + I_S)) / C_{FO}) \bullet 100 \quad [8]$$

Estimated DMD (D_E, %) of diets 3 and 4 for each horse, with the Yb internal marker, were calculated using Yb dose, I_H, I_S, and C_{FO} of Yb:

$$D_E = (1 – (\text{Yb dose} / (I_H + I_S)) / C_{FO}) \bullet 100 \quad [9]$$

Partial DMD (D_S, %) of the FF and SS supplement fed to each horse were calculated using the D_{TC} for each horse consuming orchardgrass/alfalfa hay (Diet 1) in Exp.1, individual DMD of total diet (D_T) in Exp.2, I_H, I_S, and the total diet (I_T) in Exp.2:

$$D_S = (((I_T \bullet D_T) - (I_H \bullet D_{TC})) / I_S) \bullet 100 \quad [10]$$

Fecal Kinetics - Model Development

Pooled data were used for initial model development. Fecal Cr concentrations (C_t, mg/kg DM) at time *t* (days) were determined separately for each horse (labeled 1 to 8) and daily means calculated for each diet. Diets 1 and 2, had two sets of C_t data, total collection (a) and fecal grab data (b), and each set was used in the development of the model. Diet 1a consisted of OG using the total collection data (OGTC), Diet 1b consisted of OG using the fecal grab data (OGGR), Diet 2a consisted of TF using the total collection data (TFTC), diet 2b consisted of TF using the fecal grab data (TFGR). In addition, fecal Yb concentrations (C_t, mg/kg DM) at time *t* (days) were determined separately for each horse and daily means calculated for Diets 3 and 4 in Exp.2. Diet 3Cr consisted of orchardgrass/alfalfa hay and fat-and-fiber supplement (OGFF) using the Cr external marker, and Diet 3Yb consisted of OGFF using the Yb external marker. Diet 4Cr consisted of orchardgrass/alfalfa hay and sugar-and-starch supplement (OGSS) with the Cr, and Diet 4Yb consisted of OGSS with the Yb external markers, respectively.

Daily Cr dosage for each horse was calculated using mean wt of Cr_{GB} (mg), n = 52, and mean Cr concentration (mg/kg) of Cr_{GB} :

Dose = Cr concentration (mg/kg) of Cr_{GB} • mean wt of Cr_{GB} (mg) • 3 Cr_{GB} /d [11]

Dose = 19.30 g/d

Mean daily C_t data were fitted to a single exponential, rising to an asymptote (C_a), with one rate constant (k),

$$C_t = C_a - C_a \cdot e^{-kt} \quad [12]$$

using a graphics and curve-fitting program (SlideWrite Plus 4 for Windows, Advanced Graphics Software, Carlsbad, CA). Estimates of C_a (Cr, mg/kg DM) and dose (Cr, g/d) were used to calculate daily fecal output (FO_{CA} , kg/d DM):

$$FO_{CA} = (\text{Cr dose}) / C_a \quad [13]$$

The rate constant (k, d^{-1}) was used to calculate its reciprocal, the turnover time (TT, d). The compartment size or prefecal mass (PFM, kg) was calculated from the TT and FO_{CA}:

$$TT = 1/k \quad [14]$$

$$PFM = FO_{CA} \bullet TT = FO_{CA} / k \quad [15]$$

Further development of the model included the delay between oral administration of marker and its entry into the PFM (Figure 1), following procedures for tracer kinetics (Brownell et al., 1968; Kronfeld and Ramberg, 1981). Initially, the C_t data were fit to two exponentials, but the standard errors were greater than the mean estimates of rate constants, so the data could not sustain this model. The delay (*d*, d) was represented by a decrease in time (t) in the single exponential equation:

$$C_t = C_a - C_a \cdot e^{-k(t - d)} \quad [16]$$

This equation could not be solved by the curve-fitting program, therefore a series of values of *d* from 0.1 to 0.3 d (2.4 to 7.2 h) was included in the equation, and the best fit was found to three significant figures in < 10 iterations.

The model for each diet, using the pooled data, was then applied to the separate data for each horse on each diet. The C_t data for individual horses were fitted to the exponential model with and without the delay.

Fecal recovery of Cr (R_{CR}, %) and Yb (R_{YB2}, %) for each horse offered each diet were calculated using fecal output from the total collection (FO_{TC}, kg/d DM), fecal asymptotic values (C_a), and Cr or Yb dose (dose, g/d):

$$R_{CR} = ((FO_{TC} \bullet C_a) / \text{Cr dose}) \bullet 100 \quad [17]$$

$$R_{YB2} = ((FO_{TC} \bullet C_a) / \text{Cr dose}) \bullet 100 \quad [18]$$

Adjusted fecal output (FO_{ACR} kg/d, DM) using Cr, and (FO_{AYB} kg/d, DM) using Yb for each horse offered each diet was calculated from Cr or Yb recovery instead of Cr or Yb dose:

$$FO_{ACR} = ((R_{CR1} / 100) \bullet FO_{CA}) \bullet 100 \quad [19]$$

$$FO_{AYB} = ((R_{YB1} / 100) \bullet FO_{CA}) \bullet 100 \quad [20]$$

Horses were administered Yb concurrently with Cr_{GB} allowing the pooled fecal Yb concentrations (C_t, mg/kg DM) to be used for development of a model, and C_t data were fitted to a single exponential, rising to an asymptote. The procedures used in developing the Cr based models were applied to the Yb data. This allowed comparisons to be made between the two markers.

Daily Yb dosage for each horse was calculated from the 1.500 g of $YbCl_3 \bullet 6H_2 0$ administered to each horse:

$$\text{Yb concentration in 1500 mg of } YbCl_3 \bullet 6H_2 0 = 670 \text{ mg} \quad [21]$$
$$\text{Dose} = 670 \text{ mg /d}$$

Estimated dry matter intake (DMI_E, kg/d DM) of each diet was calculated using mean Cr-predicted fecal output (FO_{CA} kg/d DM) and mean Y-predicted dry matter digestibility (D_E, %):

$$DMI_E = FO_{CA} / (1 - (D_E /100)) \quad [22]$$

Statistical Analyses

Data were summarized as means and standard errors of the mean (SEM). Significance was inferred when $P < .05$, and a trend was inferred when $P < .10$. Dependent variables (DMI, FO_{TC}, fecal concentrations of Y, Yb and Cr, and recoveries of Y, Yb and Cr, and FO_{CA}, TT and PFM) were evaluated by analysis of variance using the GLM procedure of SAS (1989) with horse and diet in the model. The Y and Yb concentrations in the diets were evaluated using the GLM procedure of SAS (1989) with diet in the model. The DMD_{TC} and DMD_E , fecal recoveries of Cr, FO_{TC}, FO_{CA}, TT, PFM, and concentrations of Cr or Yb in diurnal variation and mixed fecal grab samples of diets were evaluated using the GLM procedure of SAS (1989) with horse and method in the model.

The one-compartment model was developed using a curve-fitting program (SlideWrite Plus 4 for Windows, Advanced Graphics Software, Carlsbad, CA). Goodness of fit was determined by adjusted R-square. Simple regression analysis was used to test for linear relationships between variables. Lines of identity and paired t-tests were used to compare estimates of variables by different methods using the curve-fitting program.

Results

Experiment 1

Balance data. The DMI, FO, and D_{TC} [equation 1] for each horse offered Diets 1 and 2 are shown in Appendix table 7. Mean DMI were similar (10.2 ± .5 vs 9.4 ± .5 kg, P = .390), and mean FO were similar (3.9 ± .3 vs 3.9 ± .3 kg, P = .791) for Diet 1 and 2, respectively. Mean D_{TC} tended to be higher (P = .079) for Diet 1 (62.2 ± 1.1 %) than for Diet 2 (58.3 ± 1.2 %).

Yttrium data. Concentrations of yttrium (Y) were 1.02 ± .01 and .69 ± .02 mg/kg DM in Diets 1 and 2, respectively. Fecal concentrations of Y for horses receiving Diets 1 and 2 are shown in Appendix table 8. Mean fecal concentrations of Y were higher (P = .001) in horses fed Diet 1 (3.01 ± .17 mg/kg DM) than Diet 2 (1.81 ± .09 mg/kg DM). Fecal recoveries of Y were determined [2] for horses receiving Diets 1 and 2 (Table 1). Recoveries were similar (P = .833) for both diets, 101.3 ± 5.4 %.

The D_{TC} and estimated D_E [3] for Y of Diets 1 and 2 for each horse are shown in Table 2. The D_E were similar to D_{TC} of Diet 1 (P = .154) and Diet 2 (P = .245). Linear relationships (y = a + bx) between D_{TC} and D_E were tested for horses offered Diets 1 and 2, but were not well determined by the data for Diet 1 (F = 2.24, r = .522, P = .185) and for Diet 2 (F = 1.20, r = .408, P = .315). The intercept was not significantly different from zero and so the data were tested without the intercept (y = bx) to validate the marker method by comparison with the collection method. For Diet 1 (Figure 2) and Diet 2 (Figure 3), D_{TC} and D_E were correlated (P < .0001) for each diet, and D_E overestimated D_{TC} by 5 ± 3%. To predict D_{TC} from D_E a calibration curve of marker versus collection was tested, for Diet 1 [C = M (.94 ± .03), r = .760, P < .0001], and for Diet 2 [C = M (.94 ± .03), r = .736, P < .0001].

Fecal Kinetics Model Development

Fecal Cr concentrations (C_t) at time t (days) for each horse were used to calculate daily means for each diet (Appendix table 9 and 10). Mean daily C_t data [12] for each diet

were fitted to monoexponential curves rising to asymptotic values (Figure 4 to 7). Estimates of goodness of fit of the pooled C_t data to a one-compartment model (Figure 1), model parameters and calculated variables, in horses on each diet are shown in Table 3. All four diets in Exp.1 had good agreements (R^2 .788 to .948) between observed and predicted values ($P < .0001$, in Diets 1a, 2a and 2b, and $P = .0013$ in Diet 1b). The delays (*d*, d) were represented by a decrease in time (t) in the single exponential equation [16], and were calculated for each model (Table 4). For Diet 1b (OGGR) the post-administration curve was not fitted as well ($R^2 = .877$, $F = 14.25$, $P = .063$) as the administration curve with a delay ($R^2 = .789$, $F = 26.13$, $P = .001$), and so the administration curve with a delay model was used to represent this diet. The models for Diets 1a, 2a and 2b had improved fits (higher estimates of *F* and R^2) when a delay was included in the model (Table 4).

Individual data

Fecal Cr concentrations (C_t) at time *t* (days) were determined for each horse, offered each diet (Appendix table 11 to 14). The daily C_t for individual horses [12] on each diet were fitted to the exponential model of the respective diet with or without the delay. For each horse, the inclusion of the delay [16] improved the fit (higher estimates of *F* and R^2) of data to the one-compartment model. In some horses the post-administration curve did not fit well (lower estimates of *F* and R^2), and model variables and calculation were determined from the administration curve with a delay model. Improved fits obtained with the delay are shown in Table 5 for horses offered Diet 1a (OGTC), and for horses offered Diets 1b, 2a and 2b, the delay data are shown in Appendix tables 15 to 17.

Estimates of fit of the individual C_t to the one-compartment model and calculated variables for each horse offered Diet 1a are shown in Table 6. For horses offered Diets 1b, 2a and 2b, these data are shown in Appendix tables 18 to 20.

Comparing the Cr marker data to corresponding collection data, total collection FO_{TC} and estimates of FO_{CA} [13] from the one-compartment model for each horse were similar ($P = .354$ and $P = .514$) for Diet 1a and 2a, respectively (Table 7). Estimates of FO_{CA} tended to be higher ($P = .063$) for Diet 1a than for 2a. Linear relationships ($y = a + bx$) between FO_{TC}

and FO_{CA} were tested for horses offered Diets 1a (Figure 8) and 2a (Figure 9), and were not well determined by the data for Diet 1a (F = 2.39, r = .534, P = .173), and for Diet 2a (F = .58, r = .298, P = .473).

Comparing the Cr marker data to corresponding collection data, estimates of FO_{TC} and FO_{CA} from the one-compartment model for each horse were similar (P = .340) for Diet 1b and differed (P = .001) for Diet 2b (Table 8). Estimates of FO_{CA} were similar (P = .253) between Diet 1b and 2b. A linear relationship (y = a + bx) between FO_{TC} and FO_{CA} was found (F = 85.43, r = .966, P < .0001) for horses offered Diet 1b (Figure 10). A linear relationship (y = a + bx) was found (F = 16.74, r = .857, P < .001) between FO_{TC} and FO_{CA} for horses offered Diet 2b (Figure 11). In Diet 2b, the intercept was not significantly different from zero and so the data were tested without the intercept (y = bx) to validate the marker method by comparison with the collection method (Figure 12). The FO_{TC} and FO_{CA} were correlated (P < .0001) and FO_{CA} overestimated FO_{TC} by 18 ± 4 %. To predict FO_{TC} from FO_{CA} a calibration curve of marker versus collection was tested for Diet 2b [C = M (.83 ± .02), r = .872, P < .0001].

Turnover times (TT, h) of the prefecal mass were calculated from the one-compartment model of each diet [14], for each horse and were similar (P = .555) between Diet 1a and 2a (Table 9). The TT of the prefecal mass tended to be higher (P = .072) for Diet 1b than for Diet 2b (Table 10).

Prefecal masses (PFM, kg) were calculated from the one-compartment model of each diet [15], for each horse and were not different (P = .702) in Diets 1a and 2a (Table 11), and were also not different (P = .852) in Diets 1b and 2b (Table 12).

Fecal concentrations of Cr were determined (d 5 to d 8 of dosing) for each horse offered Diet 1a and 2a (Appendix table 21) and (d 1 to d 8 of dosing) for those offered Diet 1b and 2b (Appendix table 22). Mean fecal recoveries (d 5 to d 8 of dosing) of Cr (R_{CR}, %) were determined [17]. The R_{CR} were not different (P = .218) for horses offered Diets 1a and

2a (Table 13), and were also not different (P = .864) for horses offered Diets 1b and 2b (Table 14).

Daily Cr dosage for each horse was 19.30 g [11]. Diurnal variation in fecal concentrations of Cr was determined for individual horse fecal grab samples collected on d 8 of dosing at 0700, 1500 and 2300, and compared to corresponding combined daily fecal grab samples for Diet 1 (Table 15) and Diet 2 (Table 16). In horses offered Diet 1, the mean diurnal fecal Cr concentrations differed from corresponding combined fecal grab samples (P < .001), and the coefficient of variation was 5.44 % for the three samples taken at different times of the day and representing diurnal variation (Figure 13). In horses offered Diet 2, the mean diurnal fecal Cr concentrations differed from corresponding combined fecal grab samples (P = .002), and the coefficient of variation was 10.25 % for the three individual samples representing diurnal variation.

Experiment 2

Balance data. The DMI, FO, and D_{TC} for each horse [equation 1] offered Diets 3 and 4 are shown in Appendix table 23. Mean DMI of hay (5.42 ± .15 vs 5.31 ± .19 kg, P = .259), supplement (1.82 ± .09 vs 1.92 ± .04 kg, P = .380) and FO (2.64 ± .08 vs 2.61 ± .12 kg, P = .910) were similar for horses offered Diet 3 and 4, respectively. The mean D_{TC} was similar (P = .665) for horses offered Diet 3 (63.5 ± 1.0 %) and Diet 4 (63.9 ± 1.1 %,). Compared to the D_{TC} of the same orchardgrass/alfalfa hay (Diet 1) fed in Exp.1, the D_{TC} of Diet 3 was similar (P = .468) when FF was added (62.2 ± 1.08 vs 63.5 ± 1.0 %,), and in Diet 4 (P = .375) when SS was added (62.2 ± 1.08 vs 63.9 ± 1.1 %).

The partial DMD (Ds, %) of the FF and SS supplements were determined [10] for each horse (Table 17). The Ds of the supplements were similar (P = .703) for FF (70.9 ± 2.1 %) and SS (69.7 ± 1.9 %). The FF supplement was higher than SS in crude fat (9.6 vs 6.9 %), ADF (26.3 vs 8.7 %), and NDF (38 vs 18 %), and was lower in NSC (26 vs 56 %), respectively. Compared to the D_{TC} of orchardgrass/alfalfa hay (Diet 1) fed in Exp.1 without the addition of a supplement, the Ds of FF was higher (P = .012) than the D_{TC} of Diet 1

(70.9 ± 2.1 vs 62.2 ± 1.08 %, respectively) and the Ds of SS was also higher (P = .005) than the D_{TC} of Diet 1 (69.7 ± 1.9 vs 62.2 ± 1.1 %, respectively).

Yttrium data. Concentrations of Y were .995 ± .020 mg/kg DM in the orchardgrass/alfalfa hay, and .51 ± .02 and .55 ± .03 mg/kg DM in FF and SS supplements, respectively. Fecal concentrations of Y for horses receiving Diets 3 and 4 are shown in Appendix table 24. Mean fecal concentrations of Y were similar (P = .414) for horses offered Diet 3 (2.69 ± .12 mg/kg DM) and Diet 4 (2.93 ± .19 mg/kg DM). Fecal recoveries of Y were determined [6] for horses receiving Diets 3 and 4 (Table 18). Recoveries were similar (P = .115) in both diets, 106.3 ± 4.4 %.

The D_{TC} and estimated D_E [8] for Y of Diets 3 and 4 for each horse were compared (Table 19), and were different in Diet 3 (P = .012) and Diet 4 (P = .005). Linear relationships (y = a + bx) between D_{TC} and D_E were found for horses offered Diet 3 (F = 12.52, r = .822, P < .001) and Diet 4 (F = 11.15, r = .806, P < .001). The intercept was not significantly different from zero and so the data were tested without the intercept (y = bx) to validate the marker method by comparison with the collection method. For Diet 3 (Figure 14) and Diet 4 (Figure 15), D_{TC} and D_E were correlated (P < .0001), and D_E overestimated D_{TC} by 5 ± 1% in Diet 3, and by 8 ± 2 % in Diet 4. To predict D_{TC} from D_E a calibration curve of marker versus collection was tested, for Diet 3 [C = M (.95 ± .01), r = .843, P < .0001], and for Diet 4 [C = M (.92 ± .02), r = .851, P < .0001].

Ytterbium data. Horses were administered 670 mg of Yb daily [5], mixed thoroughly in the supplement to represent an internal marker. Fecal concentrations of Yb for horses receiving Diets 3 and 4 are shown in Appendix table 25. Mean fecal concentrations of Yb were similar (P = .763) for horses offered Diet 3 (119.9 ± 4.2 mg/kg DM) and Diet 4 (119.9 ± 3.2 mg/kg DM). Fecal recoveries of Yb (R_{YB1}, %) were determined [7] for horses receiving Diets 3 and 4 (Table 20), and were similar (P = .871) for both diets, 46.7 ± 2.1 %.

The D_{TC} and estimated D_E [9] for Yb of Diets 3 and 4 for each horse were determined (Table 21). The D_E for Yb underestimated D_{TC} by 66.1 % ($P < .001$) in Diet 3, and by 66.6 % ($P < .001$) in Diet 4.

Fecal kinetics and Model Development

Fecal Cr and Yb concentrations (C_t) at time *t* (days) for each horse were used to calculate daily means for each diet (Appendix table 26 and 27). Mean daily C_t data [12] for each diet were fitted to monoexponential curves rising to asymptotic values (Figure 16 to 19). Estimates of goodness of fit of the pooled C_t data to a one-compartment model (Figure 1), model parameters and calculated variables, in horses on each diet are shown in Table 22. All four diets in Exp.2 had good agreements (R^2 = .788 to .917) between observed and predicted values (P = .001, in Diet 3Cr, and $P < .00001$ in Diets 3Yb, 4Cr and 4Yb). The delays (*d*, d) were represented by a decrease in time (t) in the single exponential equation [16], and were calculated for each model (Table 23). For Diet 3Cr (OGFF), the data during the administration period fit the model better (R^2 = .845, F = 26.04, P = .001) than did the post-administration data (R^2 = .755, F = 9.24, P = .056), so the latter were not used in the model for this diet. Diets 3Yb, 4Cr and 4Yb had improved fits (higher estimates of F and R^2) when a delay was included in the model (Table 23).

Individual data

Fecal Cr and Yb concentrations (C_t) at time *t* (days) were determined for each horse offered each diet (Appendix table 28 to 31). The daily C_t for individual horses [12] on each diet were fitted to the exponential model of the respective diet with or without the delay. For each horse, the inclusion of the delay [16] improved the fit (higher estimates of F and R^2) of data to the one-compartment model. In some horses the post-administration data fit the model poorly ($F < 14$ and $R^2 < .85$), so only the data during the administration period were used in the model. Examples of the improved fits with the delay are shown for horses offered Diet 4Cr, using the Cr external marker (Table 24), and for horses offered Diet 4Yb, using the Yb external marker (Table 25). Improved fits with the delay for horses offered the Diets 3Cr and 3Yb using the Cr and Yb external markers, respectively, are shown in Appendix table 32 and 33.

Estimates of fit of the individual C_t to the one-compartment model and variables for each horse offered Diet 3Cr (Table 26) and 4Cr (Table 27) were calculated using Cr and Yb markers, respectively. These data are shown for horses offered Diet 3Yb (Appendix table 34) and Diet 4Yb (Appendix table 35).

Comparing the marker data to corresponding collection data, FO_{TC} and FO_{CA} estimates from the one-compartment model for each horse were different (P = .014 and P = .048) for Diet 3Cr and 4Cr, respectively (Table 28). Estimates of FO_{CA} overestimated FO_{TC} by 1.26 kg DM (32 %) for Diet 3Cr, and by 1.02 kg DM (28 %) for Diet 4Cr. Estimates of FO_{CA} were similar (P = .137) between Diet 3Cr and 4Cr. A linear relationship (y = a + bx) between FO_{TC} and FO_{CA} was tested for horses offered Diet 3Cr (Figure 20), but it was not well determined by the data (F = 1.11, r = .395, P = .218). The intercept was not significantly different from zero and so the data were tested without the intercept (y = bx) to validate the marker method by comparison with the collection method (Figure 21). The FO_{TC} and FO_{CA} were correlated (P < .0001) and FO_{CA} overestimated FO_{TC} by 47 ± 7 %. To predict FO_{TC} from FO_{CA} a calibration curve of marker versus collection was tested for Diet 3Cr [C = M (.66 ± .03), r = .721, P < .0001].

Adjusted estimates of FO_{CA} [19] calculated from Cr recovery, instead of dose (FO_{ACR}), slightly improved the linear relationship (Figure 22) for Diet 3Cr (smaller SE and 95 % confidence intervals), but considerably improved the correlation (P < .0001) of FO_{TC} and FO_{CA}, and estimates of FO_{ACR} were equal to FO_{TC} values (Figure 23). A linear relationship (y = a + bx) between FO_{TC} and FO_{CA} was tested for horses offered Diet 4Cr (Figure 24), but was not well determined by the data (F = .11, r = .135, P = .800).

Comparing the marker data to corresponding collection data, FO_{TC} and FO_{CA} estimates from the one-compartment model for each horse were different (P = .0002 and P = .0002) for Diets 3Yb and 4Yb, respectively (Table 29). Estimates of FO_{CA} overestimated the FO_{TC} by 1.94 kg DM (42 %) for Diet 3Yb, and overestimated by 1.8 kg DM (40 %) for Diet 4Yb. Estimates of FO_{CA} were similar (P = .084) between Diets 3Yb and 4Yb. A linear relationship (y = a + bx) between FO_{TC} and FO_{CA} was tested for horses offered Diet 3Yb

(Figure 25), but was not well determined by the data (F = 1.01, r = .379, P = .083). The intercept was not significantly different from zero and so the data were tested without the intercept (y = bx) for identity, to validate the marker method by comparison with the collection method (Figure 26). The FO_{TC} and FO_{CA} were correlated ($P < .0001$), and FO_{CA} overestimated FO_{TC} by 73 ± 5 %. To predict FO_{TC} from FO_{CA} a calibration curve of marker versus collection was tested for Diet 3Yb [C = M (.57 ± .02), r = .611, $P < .0001$].

Adjusted estimates of FO_{CA} [20] calculated from Yb recovery, instead of dose (FO_{AYB}), slightly improved the linear relationship (Figure 27) for Diet 3Yb (smaller SE and 95 % confidence intervals), but considerably improved the correlation ($P < .0001$) of FO_{TC} and FO_{CA}, and estimates of FO_{AYB} were virtually equal to FO_{TC} values (Figure 28). A linear relationship (y = a + bx) between FO_{TC} and FO_{CA} was tested for horses offered Diet 4Yb (Figure 29), but was not well determined by the data (F = .15, r = .158, P = .374).

Comparing the Cr and Yb external marker estimates of FO_{CA} , Diets 3Cr and 3Yb were different (P = .027), and Yb marker FO_{CA} overestimated Cr values by .68 kg DM (14.8 %). For Diets 4Cr and 4Yb, estimates of FO_{CA} predicted by Cr and Yb were also different (P = .0001), and Yb marker FO_{CA} overestimated Cr values by .77 kg DM (17.6 %). Estimates of FO_{CA} differed (P = .0001) for Diets 3Cr and 4Cr combined and marked by Cr, compared to Diets 3Yb and 4Yb combined and marked by Yb. The Yb marker estimates were 1.46 kg DM (16.2 %) higher than Cr marker estimates.

Comparing Diet 1a (orchardgrass/alfalfa hay) fed in Exp.1 and Diet 3Cr (OG plus FF) fed in Exp.2 using the Cr marker, the FO_{CA} estimates were similar (P = .116), and when Diet 1a fed in Exp.1 was compared to Diet 4Cr (OG plus SS) in Exp.2, the FO_{CA} estimates differed (P = .013). Estimates of FO_{CA} for horses offered the hay only (Diet 1a) were .484 kg DM (11.7 %) higher than for the hay and SS supplement (Diet 4Cr). The estimated FO_{CA} of the main one-compartment models for each diet in Exp.1 and 2, were similar (P = .838) to the mean estimated FO_{CA} of the individual horses for all eight diets.

Turnover times (TT, h) of the prefecal mass were calculated [14] from model for each horse and were similar (P = .356) for Diets 3Cr and 4Cr (Table 30). The TT of the prefecal mass were also similar (P = .928) for Diets 3Yb and 4Yb (Table 31). Comparing the TT calculated using either Cr or Yb markers, the TT of Diets 3Cr and 3Yb were similar (P = .502). A linear relationship (y = a + bx) was tested between Cr-predicted TT and Yb-predicted TT (F = 4.63, r = .660, P = .075) for horses offered Diet 3Cr and 3Yb.

Comparing TT calculated using either Cr or Yb markers, the TT of Diet 4Cr and 4Yb were similar (P = .554). A linear relationship (y = a + bx) between Cr-predicted TT and Yb-predicted TT was tested for horses offered Diet 4Cr and 4Yb, but was not well determined by the data (F = .86, r = .354, P = .388). The intercept was not significantly different from zero and so the data were tested without the intercept (y = bx) to compare the two marker methods. The TT of Diet 4Cr and 4Yb were correlated [Cr TT = Yb TT (.95 ± .05), P < .001], so Yb estimates of TT were 5 ± 5 % lower than Cr values of TT.

Comparing diets, the Cr-predicted TT were similar (P = .444) in (OG hay) fed as Diet 1a in Exp.1 and Diet 3Cr (OG hay plus FF), but differed (P = .025) between Diet 1a and Diet 4Cr (OG hay plus SS). The TT of Diet 1a were 16.8 % lower than in Diet 4Cr. The TT of the main models for each diet in Exp.1 and 2 were similar (P = .114) to the means of the TT of the individual horses.

Prefecal masses (PFM) were calculated [15] for each horse and were similar (P = .864 in Diets 3Cr and 4Cr (Table 32), and similar (P = .581) in Diets 3Yb and 4Yb (Table 33). Using either Cr or Yb markers, the PFM was lower (P = .036) in Diet 3Cr than in Diet 3Yb. A linear relationship (y = a + bx) between Cr-predicted PFM and Yb-predicted PFM was found (F = 32.66, r = .919, P = .001) for horses offered Diets 3Cr and 3Yb (Figure 30).

Using either Cr or Yb markers, the PFM were lower (P < .001) in Diet 4Cr than in Diet 4Yb. A linear relationship (y = a + bx) between Cr-predicted PFM and Yb-predicted PFM was tested for horses offered Diet 4Cr and 4Yb, but was not well determined by the data (F = .55, r = .291, P = .484). The intercept was not significantly different from zero

and so the data were tested without the intercept (y = bx) to compare the two marker methods (Figure 31). The PFM of Diet 4Cr and 4Yb were correlated ($P < .001$), and Yb estimates of PFM were 15 ± 6 % higher than Cr values of PFM.

Comparing diets, the Cr-predicted PFM were similar ($P = .688$) for Diet 1a (OG hay) fed in Exp.1, and for Diet 3Cr (OG hay plus FF) fed in Exp.2, and were also similar ($P = .335$) in Diets 1a and Diet 4Cr (OG hay plus SS). The PFM of the main models for each diet in Exp.1 and 2 were similar ($P = .172$) to the means of the PFM of the individual horses.

Fecal concentrations of Cr were determined (d 1 to d 8 of dosing) for each horse offered Diet 3Cr and 4Cr (Appendix table 36). Fecal concentrations of Yb were determined (d 1 to d 8 of dosing) for each horse offered Diet 3Yb and 4Yb (Appendix table 37). Mean fecal recoveries (d 1 to d 8 of dosing) of Cr (R_{CR}, %) were determined [17]. The R_{CR} were similar ($P = .570$) for horses offered Diet 3Cr and 4Cr, 70 ± 2.9 % (Table 34). Mean fecal recoveries (d 1 to d 8 of dosing) of Yb (R_{YB2}, %) were determined [18]. The R_{YB2} were similar ($P = .883$) for horses offered Diet 3Yb and 4Yb, 58.9 ± 2.3 % (Table 35).

Daily Cr dosage for each horse was 19.30 g [11]. Diurnal variation in fecal concentrations of Cr was determined for individual horse fecal grab samples collected on d 8 of dosing at 0700, 1500 and 2300, and compared to corresponding combined daily fecal grab samples for Diet 3Cr (Appendix table 38) and Diet 4Cr (Appendix table 39). In horses offered Diet 3Cr, the mean diurnal fecal Cr concentrations were higher ($P < .001$), than corresponding combined fecal grab samples, and the coefficient of variation was 8.47 % for the three individual samples representing diurnal variation. In horses offered Diet 4Cr, the mean diurnal fecal Cr concentrations were higher ($P < .001$) than corresponding combined fecal grab samples, and the coefficient of variation was 6.27 % for the three individual samples representing diurnal variation (Figure 32).

Daily Yb dosage for each horse was 670 mg [21]. Diurnal variation in fecal concentrations of Yb was determined for individual horse fecal grab samples collected on d

8 of dosing at 0700, 1500 and 2300, and compared to corresponding combined daily fecal grab samples for Diet 3Yb (Appendix table 40) and Diet 4Yb (Appendix table 41). In horses offered Diet 3Yb, the mean diurnal fecal Yb concentrations were higher ($P < .001$) than corresponding combined fecal grab samples, and the coefficient of variation was 9.56 % for the three individual samples representing diurnal variation. In horses offered Diet 4Yb, the mean diurnal fecal Yb concentrations were higher ($P < .001$) than corresponding combined fecal grab samples ($P < .001$), and the coefficient of variation was 8.08 % for the three individual samples representing diurnal variation (Figure 33).

Dry matter percentage (DM, %) of feces for horses offered each diet in Exp.1 and 2 are shown in Table 36. The DM of feces were similar ($P = .524$) for horses offered Diet 1 and 2, but differed ($P = .002$) between horses offered Diet 3 and 4. The DM of feces were different ($P < .001$) between Diet 1 (OG hay only) and Diet 3 (OG hay plus FF supplement), and also differed ($P = .001$) between Diet 1 and Diet 4 (OG hay plus SS supplement). Mean DM of feces of Diet 1 was 4.65 % lower than Diet 3, and 6.43 % lower than Diet 4. Mean DM of feces of Diet 3 was 1.78 % lower than Diet 4.

A linear relationship ($y = a + bx$) was tested between fecal DM and Cr-predicted TT for horses offered Diet 1 (OG hay only) and Diet 3Cr (OG plus FF supplement) in Exp.1 and 2, respectively (Figure 34), but was not well determined by the data ($F = 2.44$, $r = .385$, $P = .140$). The intercept was not significantly different from zero and so the data were tested without the intercept (Figure 35). The fecal DM and TT for Diet 1 and 3Cr were correlated ($F = 6.77$, $r = .56$, $P = .021$), and fecal DM can be used to predict TT [fecal DM = TT ($.99 \pm .04$)].

A linear relationship ($y = a + bx$) was tested between DM of feces and Cr-predicted TT for horses offered Diet 1 (OG hay only) and Diet 4Cr (OG plus SS supplement) in Exp.1 and 2, respectively (Figure 36), but was not well determined by the data ($F = 7.52$, $r = .591$, $P = .016$) and SE was larger than the mean estimate of the intercept. The intercept was not significantly different from zero, so the data were tested without the intercept (Figure 37).

The fecal DM and TT for Diet 1 and 4Cr were correlated (F = 12.75, r = .68, P = .003), and fecal DM can be used to predict TT [fecal DM = TT (1.01 ± .04)].

A linear relationship (y = a + bx) was found (F = 13.14, r = .701, P = .002) between fecal DM and Yb-predicted TT for horses offered Diet 1 (OG hay only) and Diet 3Yb (OG plus FF supplement) in Exp.1 and 2, respectively (Figure 38). The intercept was not significantly different from zero, so the data were tested without the intercept (Figure 39). The fecal DM and TT for Diet 1 and 3Yb were correlated (F = 18.06, r = .740, P < .001) and fecal DM of feces can be used to predict TT [fecal DM = TT (1.02 ± .02)].

A linear relationship (y = a + bx) was found (F = 5.41, r = .53, P = .036) between fecal DM and Yb predicted TT for horses offered Diet 1 (OG hay only) and Diet 4Yb (OG plus SS supplement) in Exp.1 and 2, respectively (Figure 40). The intercept was not significantly different from zero and so the data were tested without the intercept (Figure 41). The fecal DM and TT for Diet 1 and 4Yb were correlated (F = 13.7, r = .70, P = .002) and fecal DM can be used to predict TT [fecal DM = TT (.98 ± 03)].

A linear relationship was found (F = 19.87, r = .88, P = .002) between mean fecal DM and mean TT for all horses fed hay only in Exp.1, or hay plus supplement in Exp.2 (Figure 42).

Mean DMI estimates [22] for horses offered each diet were determined using mean Cr-predicted FO_{CA} and mean Y-predicted D_E. The DMI_E for Diets 1 and 2 were 10.2 and 9.4 kg/d DM, respectively. The DMI_E overestimated balance DMI by 13 % in Diet 1, and 3 % in Diet 2. The DMI_E for Diets 3 and 4 was 11.7 kg/d DM, and overestimated balance DMI by 38 %, for each diet.

Discussion

The results showed that fecal Cr kinetics could be calibrated precisely (SE 1 to 3 %) to predict fecal DM output of horses fed orchardgrass/alfalfa hay, tall fescue/alfalfa hay, OG hay plus fat-and-fiber supplement, but not OG hay plus sugar-and-starch supplement. Similarly, fecal Yb kinetics were calibrated to predict fecal DM ouput of horses fed OG hay plus FF supplement, but not OG hay plus SS supplement. Moreover, concentrations of Y in the feed and feces estimated digestibility precisely (SE 1 to 3 %). Thus, a combination of theses two methods could be used to estimate DM intake. The method of dosing three times a day diminished diurnal variation, but discrepancies between Cr and Yb concentration means of diurnal samples and combined samples showed incomplete mixing, the major source of tracer error. Therefore more frequent administration and fecal samples should be tested in future experiments.

Experiment 1

Balance data. The D_{TC} of tall fescue/alfalfa mixed hay of Diet 2 (58.3 %) was similar to a previous study where total collection DMD estimates of 58 % and 48 % were observed for horses fed mid-bloom alfalfa and tall fescue hay, respectively (Crozier, et al., 1997). The NDF content in Diet 2 was 5 % higher than in Diet 1 and may account for the slightly lower D_{TC} of Diet 2, since NDF is negatively correlated with DMD in ruminants (Minson, 1990). In other studies where total collection methods were used, D_{TC} estimates of 61 % for alfalfa hay harvested at 1/10 bloom, and 50 % for orchardgrass hay (early stage of maturity) were observed (Vander Noot and Gilbreath, 1970). The D_{TC} of alfalfa:orchardgrass (80:20) mixed hay in horses decreased from 69.1 % (pre-bloom), to 57.3 (mid-bloom), and in orchardgrass hay, the D_{TC} decreased from 63.1 to 54.7 % (Darlington and Hershberger, 1968). The higher D_{TC} of orchardgrass/alfalfa mixed hay of Diet 1 (62.2 %) observed in this study corresponds to the higher D_{TC} of hays when harvested at earlier stages of maturity (Darlington and Hershberger, 1968).

Marker Digestibility. Yttrium (Y) has not been used previously as a marker in horses. In chicks, a single dose of an isotope (^{91}Y) resulted in >95 % recoveries (Sklan et

al., 1975). In both Diet 1 and 2 the relationship between D_{TC} and D_E indicated a high level of accuracy (5 % overestimation) and precision (SE ± 5 %), validating the use of Y in estimating digestibility of hay diets in horses.

Fecal Kinetics. The present results confirm the use of a one-compartment model with a delay to describe fecal kinetics in the horse using Cr (Holland et al., 1998). The model also fits Yb kinetics. Previous studies of two-compartment systems in sheep, cattle, and horses have combined marker methods for estimating transit time and mean retention time with kinetic estimates of rate constants. In some models, an age-dependent (time-dependent) rate function that substitutes for one rate constant has been used (Blaxter et al., 1956; Grover and Williams, 1973; Pond et al., 1988; Bertone et al., 1989). In many of these studies, the SE of rate constants have not been published, and in other studies, large SE relative to rate constants have not led to rejection of the model.

In this study, a two-compartment model was tested and rejected because the SE were greater than the mean estimates of rate constants for the second compartment, in other words, the data could not sustain this model. One reason that may account for not being able to determine a second mixing compartment could be the simpler digestive tract of horses. In ruminants, the rumen-reticulum serves as an initial mixing chamber, which complicates kinetic analysis, and is absent in horses. In the one-compartment model used in this study, the delay represents the movement of the oral dose of marker from the mouth to the PFM without any appreciable mixing. The present estimates of 3 to 6 h are longer than the delay of 2 to 3 h found previously (Holland et al., 1998). The present estimates may more realistically relate to mouth-to-cecum transport times, because the marker was administered three times a day instead of once, and the initial part of the tracer curve was more precisely defined.

The models used in this study assume that the chromic oxide (Exp.1 and 2) and ytterbium chloride (Exp.2) are not mixing in the delay component, which is between the mouth and the PFM. In order to estimate digestibility, thorough mixing of the markers throughout the small intestine to ensure an even distribution of the markers throughout the

feed is necessary, for example, by mordanting Cr to fiber (Uden et al., 1980), or by the soak and rinse method for Yb (Pond et al., 1988).

The main assumption in the development of the models in this study was that chromic oxide and ytterbium chloride particles are mixing thoroughly in a prefecal mass of material consisting of water with potentially dissolved or suspended DM. The feces are removed from this material and represent the samples. The PFM is assumed to be mainly in the large colon and cecum, but it is determined by marker dilution and may not necessarily represent exactly the contents of the large colon and cecum, which ranges from 3 to 7 kg of DM in horses (Alexander, 1972; Bertone et al., 1989).

Comparing the model parameters and calculated variables generated from the one-compartment models in this study to a similar previous study in horses (Holland et al., 1998), the FO_{CA}, were slightly higher in this study, from 3.7 to 4.5 kg/d, for the four hay diets versus 3.4 kg/d for the hay diet in the previous study. The PFM were slightly lower in this study, from 3.6 to 4.2 kg DM for the four hay diets versus 4.6 kg in the previous study. The TT were considerably lower in this study, from 21.4 to 25.4 h vs 33 h in the previous study. In this study, the rate constant was determined from both the adminstration and post-administration curves. In two of the models and for a few individual horses, the rate constant of the post-administration curve was not well determined by the data, suggesting that improvements may be obtained with more frequent fecal sample collection, during the post-administration period in future experiments.

Chromic oxide was used in another study to estimate mean retention time (MRT), and was 38 h in horses fed alfalfa hay (Vander Noot et al., 1967). Estimates of MRT of hays using other markers have ranged from 23 to 36 h (Orton et al., 1985). The MRT estimates are usually based on marker appearance in feces (Blaxter et al., 1956) which correspond approximately to the delay plus the TT of the models developed in this study. The average TT and delay of the four hay diets in this study were 23 h for TT and 4.95 h delay, and so the estimated MRT of 27.9 h would correspond to the MRT of hay diets found in these other studies.

The means of the three individual fecal grab samples representing diurnal variation were significantly different from corresponding combined daily grab samples. This finding was consistent in both Diet 1 and 2, and it is difficult to explain. The error appears to be systematic in that, the degree of error is similar for each diet. Coefficients of variation for Diets 1 and 2 were 5 and 10 %, considerably less than the 79 % found previously (Holland et al., 1998). This improvement can be explained by the method of marker administration. In the previous study, chromic oxide was administered once daily and the authors suggested that more frequent dosing would improve model estimates of fecal output. In this study, the marker was administered three times a day at 8 h intervals and showed that the more frequent dosing of marker reduces the diurnal variation of fecal Cr concentration. In addition, further improvement in mixing would be expected with more frequent dosing.

Experiment 2

Balance data. The D_{TC} of the orchardgrass/alfalfa hay plus FF supplement (Diet 3) was similar to the D_{TC} of the OG hay plus SS supplement (Diet 4) and both Diets 3 and 4 were similar in D_{TC} to the OG hay (Diet 1) in Exp.1. The DMI of hay was 47 % lower in Exp.2 than in Exp.1. Digestibility is usually increased with the addition of supplements to the diet, and so this result was surprising. Increased digestibility of NSC, which occurs mainly in the small intestine, was most likely counteracted by the decreased digestibility of fiber because of the reduced hay intake.

The partial digestibility of the supplements was similar between FF and SS supplements, and the FF supplement was higher in ADF and NDF than the SS supplement. The higher ADF and NDF content of the FF supplement did not affect the D_{TC} between Diets 3 and 4 or the partial digestibility of FF and SS. More generally, higher ADF and NDF contents are negatively correlated with DMD (Minson, 1990).

Marker Digestibility. In both Diet 3 and 4, linear relationships were found between D_{TC} and D_E with high levels of accuracy and precision, validating the use of Y in estimating digestibility of combined hay and supplement diets in horses.

Fecal recoveries of Yb were very low (< 50 %) in both Diet 3 and 4 and account for the large underestimation of digestibility of these two diets. For example, in one horse, the fecal recovery of Yb was 53.2 % and the D_E of Yb for its diet was 30 %, and when the recovery is substituted for the dose (Blaxter et al., 1956; Titgemeyer, 1997) the D_E is virtually equal to D_{TC}. Effectively, 53 % of the dose was not recovered and this amount failed to mix and contribute to the calculation of D_E.

Fecal Kinetics. The one-compartment model with a delay yielded good fits to the data using both Cr and Yb markers in Exp.2. The delays were 1.3 h less in the Yb model of Diet 3 than the Cr model, and 1.5 h less in the Yb compared to the Cr models of Diet 4. The delays introduced to the Cr model for both diets do not correspond to the previous study (Holland et al., 1998), where a 2 h delay gave the best fit in the model for horses fed hay and supplement versus 6 to 7 h for similar diets in this study. The present estimates may more realistically relate to mouth-to-cecum transport times, because the marker was administered three times a day instead of once, and the initial part of the tracer curve was more precisely defined. The longer delays in Exp.2 compared to Exp.1 may be on account of the longer period required for the supplement to be digested in the small intestine than the hay only diet, which would remain in the large intestine for a longer period during fermentation.

Comparing the model parameters and calculated variables generated from the models of Diet 3 and 4 (Cr data only) in this study, to the hay and supplement model used by Holland et al., (1998), the FO_{CA} were similar, 3.7 and 3.5 kg/d, for Diet 3Cr and 4Cr versus 3.8 kg/d in the previous study. The PFM were considerably higher in this study, with 3.5 and 4.2 kg DM for Diet 3Cr and 4Cr versus 2.9 kg DM found previously. The TT were higher in this study, with 22.6 and 29.4 h (Diets 3Cr and 4Cr, respectively) vs 18.1 h for the hay plus supplement diet in the previous study.

Recoveries of Cr approached 100 % in Exp.1, using hay only, but were much lower in Exp.2 using hay plus supplements. When marker estimates were adjusted for Cr recovery, an improved relationship between marker and collection values was observed, and

showed that if recoveries were accounted for, the marker estimates were equal to collection values.

The recovery of Yb was low in both Diet 3Yb and 4Yb, and marker estimates of FO substantially overestimated collection values. When marker estimates were adjusted for fecal Yb recovery in Diet 3Yb, the marker estimates were virtually equal to collection data. The individual horse values of FO corresponded to the models of the eight diets, and validate their use in estimating FO in horses.

The Cr fecal kinetics were calibrated to predict fecal DM output of horses fed OG hay, TF hay and OG hay plus FF supplement, but not OG plus SS supplement. Also, Yb fecal kinetics were calibrated to predict fecal DM output of horses fed OG plus FF, but not OG plus SS diet. An explanation may be that, in contrast to the SS supplement, the FF supplement has a high fiber content, and is similar in form to the hay only diets, which may allow more thorough mixing of marker with the digesta.

The Cr and Yb markers were not comparable for estimating FO, with Yb overestimating Cr values in both Diet 3 and 4. The TT estimated by Cr and Yb were similar for Diet 3 and 4. Diets 4Cr and 4Yb were correlated and Yb estimates of TT were $5 \pm 5\%$ lower than Cr estimates. The individual horse results for TT were similar to the eight models and show that the models can be used to predict TT in horses. Chromium and Yb estimates of PFM were not similar, but were correlated. The overestimations by the Yb marker are most likely because of the poor Yb recoveries in both diets.

Diurnal variations in Exp.2 for Cr were similar to those in Exp.1 and considerably improved from the diurnal variation observed when Cr was administered once daily (Holland et al., 1998). Diurnal variations for Yb had similar coefficients of variation to Cr values, and further improvement in mixing would be expected with more frequent dosing.

The longer TT found with hay and supplement diets, than with hay alone contrasts with previous findings in the horse (Holland et al., 1998). The present results are similar

however, to slower rates of marker excretion in sheep fed concentrates instead of all-roughage diets (Grovum and Williams, 1973). A possible explanation is that a lower fiber content retards the rate of propulsion of digesta through the digestive tract.

Implications

A model of fecal kinetics is being developed using chromic oxide or ytterbium chloride as a marker or tracer of dry matter. Experimental tests in stall-fed horses revealed systematic error in recovery of marker but reasonable precision, so that marker estimates of fecal output could be calibrated to predict total collection of feces per day. Yttrium was tested as an internal marker, and the estimates of digestibility correlated with the balance data. Diurnal variation of fecal concentrations of markers suggested that further improvements in these methods may be obtained by marker administration more than three times a day to achieve thorough mixing more rapidly in the digesta. In future experiments, more frequent fecal sample collection during the post-administration period may improve determination of the rate constant. These findings have shown that simultaneous use of chromic oxide, as the external marker, and yttrium, as the internal marker, should be tested in grazing horses. Estimates of fecal output and digestibility allow calculation of dry matter intake, which is an important variable in studies of pasture. The kinetic model also allows estimation of the prefecal mass and its turnover time, which may be important in the endurance or event horse.

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Table 1. Fecal recovery of yttrium (R_Y, %) for horses offered orchardgrass/alfalfa hay (Diet 1) and tall fescue/alfalfa hay (Diet 2) in Experiment 1[a]

Horse	Diet 1	Diet 2
1	108.6	114.0
2	85.8	84.2
3	110.2	109.9
4	105.6	109.4
5	107.8	110.4
6	77.3	73.9
7	126.4	112.7
8	95.6	91.1
Mean ± SE[b]	102.1 ± 5.4[c]	100.6 ± 5.4[c]

[a] Mean of Periods 1 and 2
[b] Standard error of the mean (n = 8)
[c] (*P* = .883)

Table 2. Total collection DMD (D_{TC}, %) and estimated DMD (D_E, %) for yttrium, for horses offered orchardgrass/alfalfa hay (Diet 1), and tall fescue/alfalfa hay (Diet 2) in Experiment 1

Horse	Diet 1		Diet 2	
	D_{TC}	D_E	D_{TC}	D_E
1	61.91	67.85	60.37	68.11
2	66.97	64.37	53.97	49.83
3	65.03	70.90	59.95	66.60
4	60.78	65.93	56.06	63.18
5	59.84	65.83	57.39	64.59
6	61.37	54.11	64.06	55.42
7	64.90	74.53	60.12	67.54
8	57.49	59.21	54.65	54.32
Mean ± SE[a]	62.3 ± 1.1[b]	65.3 ± 2.2[b]	58.3 ± 1.2[c]	61.2 ± 2.4[c]

[a] Standard error of the mean (n = 8)
[b] (P = .154); [c] (P = .245)

Table 3. Estimates of fit of pooled fecal chromium concentration data to a one-compartment model, model parameters, and calculated variables in horses offered orchardgrass/alfalfa hay using total collection data (Diet 1a), or fecal grab data (Diet 1b), and tall fescue/alfalfa hay using total collection data (Diet 2a), or fecal grab data (Diet 2b), in Experiment 1[a]

Estimate	Diet 1a OGTC	Diet 1b OGGR	Diet 2a TFTC	Diet 2b TFGR
R^2	.876	.788	.948	.862
F	77.73	26.13	202.5	68.44
P-value	.00000	.00138	.00000	.00000
Cr_a, mg/kg[b]	4.932	4.391	5.103	4.227
SE[c]	.434	.522	.247	.371
t-statistic	11.351	8.407	20.635	11.385
P-value	.00001	.00007	.00000	.00001
k, d^{-1d}	.945	1.121	1.025	1.093
SE	.321	.448	.183	.295
t-statistic	3.295	1.989	5.308	3.095
P-value	.01320	.08701	.00111	.01743
TT, h[e]	25.40	21.40	23.40	21.95
Delay, h[f]	5.592	6.192	3.144	4.872
FO_{CA}, kg/d[g]	3.913	4.395	3.782	4.565
PFM, kg[h]	4.140	3.920	3.688	4.176

[a] Pooled data = mean of eight horses; [b] Cr_a is the asymptotic value of fecal Cr concentration
[c] Standard error of the mean (n = 9); [d] k is the rate constant
[e] TT is turnover time of the prefecal mass, TT = -1/k
[f] Delay is the time between the administration of Cr and its entry into the prefecal mass
[g] FO_{CA}, is the fecal output, FO_{CA} = dose of Cr/Cr_a; [h] PFM is prefecal mass or mixing compartment, PFM = FO_{CA} • TT.

Table 4.

Fits of data to a one-compartment model of fecal kinetics without a delay, the administration curve, the post-administration curve, and the total curve with a delay between administration of an oral dose of Cr and its entry into the compartment, in horses offered orchardgrass/alfalfa hay using total collection data (Diet 1a) or fecal grab data (Diet 1b), or tall fescue/alfalfa hay using total collection data (Diet 2a), or fecal grab data (Diet 2b), in Experiment 1[a].

	No delay			Administration curve			Post-Administration Curve			Total Curve		
Diet	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value
Diet 1a OGTC[b]	.782	25.06	.0016	.844	37.98	.0005	.909	19.91	.0467	.876	77.73	.0000
Diet 1b OGGR[c]	.697	16.13	.0051	.789	26.13	.0014	.877	14.25	.0636	.829	53.14	.0000
Diet 2a TFTC[d]	.922	82.56	.0000	.939	107.86	.0000	.986	136.61	.0072	.948	202.57	.0000
Diet 2b TFGR[e]	.751	21.12	.0025	.817	31.16	.0008	.950	37.61	.0256	.862	68.44	.0000

[a] Pooled data for eight horses
[b] Delays were calculated for each model: [b] (5.592 h); [c] (6.192 h); [d] (3.144 h); [e] (4.872 h)

Table 5.

Fits of data to a one-compartment model of fecal kinetics without a delay, the administration curve with a delay, the post-administration curve, and the total curve with a delay between administration of an oral dose of Cr and its entry into the compartment, in horses offered orchardgrass/alfalfa hay using total collection data in Experiment 1[a, b, c]

	No Delay			Administration Curve			Post-Administration Curve			Total Curve		
Horse	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value
1	.736	19.56	.0031	.818	31.42	.0008	.613	3.17	.2169	.827	52.65	.0000
2	.515	7.42	.0296	.640	12.43	.0097	.835	10.15	.0860	.737	30.79	.0002
3	.548	8.48	.0226	.669	14.13	.0071	.532	2.28	.2703	.693	24.82	.0004
4	.723	18.24	.0037	.795	27.13	.0012	.943	33.39	.0287	.797	43.10	.0000
5	.639	12.41	.0097	.736	19.56	.0031	.968	60.14	.0162	.778	38.60	.0001
6	.879	50.98	.0002	.885	53.66	.0002	.952	39.70	.0243	.903	101.99	.0000
7	.591	10.10	.0155	.713	17.36	.0042	.452	1.65	.3279	.697	25.26	.0004
8	.594	10.24	.0151	.708	17.00	.0045	.959	46.39	.0209	.748	32.63	.0001

[a] Individual horse data
[b] Delay from mean OGTC model (5.592 h)
[c] Model fitted using the chromium external marker

Table 6. Estimates of fit of individual fecal chromium concentration data to a one-compartment model, model parameters, and calculated variables in horses offered orchardgrass/alfalfa hay, using total collection data (Diet 1a) in Experiment 1

Horse	Cr_a, mg/kg[a]	FO_{CA}, kg/d[b]	TT, h[c]	PFM, kg[d]	R^2	*F*	*P*-value
1	5.082	3.797	25.25	3.996	.818	31.42	.00081
2	4.825	3.999	23.37	3.895	.737	30.79	.00086
3	4.654	4.146	22.68	3.920	.669	14.13	.00709
4	4.313	4.474	23.94	4.464	.797	43.10	.00031
5	4.333	4.453	18.55	3.442	.778	38.60	.00044
6	5.610	3.440	27.39	3.926	.903	101.99	.00002
7	4.966	3.885	20.81	3.370	.713	17.36	.00421
8	4.048	4.767	18.03	3.582	.748	32.63	.00073

[a] Cr_a is the asymptotic value of fecal Cr concentration
[b] FO_{CA} is the fecal output, FO_{CA} = dose of Cr/Cr_a
[c] TT is turnover time of the prefecal mass, TT = -1/k
[d] PFM is prefecal mass or mixing compartment, PFM = $FO_{CA} \bullet$ TT

Table 7. Total collection fecal output (FO_{TC}, kg/d DM), and estimates of fecal output (FO_{CA}, kg/d DM) from the one-compartment model, using total collection Cr data for horses offered orchardgrass/alfalfa hay (OGTC, Diet 1a) and tall fescue/alfalfa hay (TFTC, Diet 2a), in Experiment 1[a]

	Diet 1	Diet 1a	Diet 2	Diet 2a
	OG	OGTC	TF	TFTC
Horse	FO_{TC}	FO_{CA}	FO_{TC}	FO_{CA}
1	3.83	3.79	3.80	3.98
2	3.25	3.99	4.39	3.43
3	2.97	4.14	2.74	3.34
4	4.70	4.47	4.23	4.51
5	4.56	4.45	4.73	4.08
6	4.12	3.44	3.96	3.23
7	2.71	3.88	3.01	3.84
8	4.84	4.76	4.68	3.71
Mean ± SE[b]	3.90 ± .29[c]	4.12 ± .15[c,e]	3.90 ± .31[d]	3.76 ± .15[d,e]

[a] FO_{CA} = dose of Cr/C_a (asymptote)
[b] Standard error of the mean (n = 8)
[c] (*P* = .354); [d] (*P* = .514); [e] (*P* = .063)

Table 8. Total collection fecal output (FO_{TC}, kg/d DM), and estimates of fecal output (FO_{CA}, kg/d DM) from the one-compartment model, using fecal grab Cr data for horses offered orchardgrass/alfalfa hay (OGGR, Diet 1b) and tall fescue/alfalfa hay (TFGR, Diet 2b), in Experiment 1[a]

	Diet 1	Diet 1b	Diet 2	Diet 2b
	OG	OGGR	TF	TFGR
Horse	FO_{TC}	FO_{CA}	FO_{TC}	FO_{CA}
1	3.83	4.20	3.80	4.45
2	3.25	3.72	4.39	5.45
3	2.97	3.47	2.74	3.46
4	4.70	5.12	4.23	5.67
5	4.56	4.81	4.73	5.22
6	4.12	4.94	3.96	4.02
7	2.71	3.59	3.01	4.02
8	4.84	3.32	4.68	5.27
Mean ± SE[b]	3.90 ± .29[c]	4.40 ± .26[c,e]	3.90 ± .26[d]	4.69 ± .28[d,e]

[a] FO_{CA} = dose of Cr/C_a (asymptote)
[b] Standard error of the mean (n = 8)
[c] (P = .340); [d] (P = .001); [e] (P = .253)

Table 9. Turnover times (TT, h) from the chromium one-compartment model, using total collection data, for horses offered orchardgrass/alfalfa hay (OGTC, Diet 1a) and tall fescue/alfalfa hay (TFTC, Diet 2a) in Experiment 1[a]

	Diet 1a	Diet 2a
Horse	OGTC	TFTC
1	25.25	20.36
2	23.37	35.14
3	22.68	32.52
4	23.94	16.38
5	18.55	18.89
6	27.39	21.94
7	20.81	23.00
8	18.03	24.34
Mean ± SE[b]	22.50 ± 1.14[c]	24.07 ± 2.31[c]

[a] TT is turnover time of the prefecal mass, TT = -1/k
[b] Standard error of the mean (n = 8)
[c] (*P* = .555)

Table 10. Turnover times (TT, h) from the chromium one-compartment model, using fecal grab data, for horses offered orchardgrass/alfalfa hay (OGGR, Diet 1b) and tall fescue/alfalfa hay (TFGR, Diet 2b) in Experiment 1[a]

	Diet 1b	Diet 2b
Horse	OGGR	TFGR
1	23.33	21.53
2	24.55	18.18
3	28.08	32.28
4	22.64	16.87
5	19.07	17.77
6	17.68	18.11
7	20.04	21.42
8	18.22	19.97
Mean ± SE[b]	21.70 ± 1.27[c]	20.51 ± 1.78[c]

[a] TT is turnover time of the prefecal mass, TT = -1/k
[b] Standard error of the mean (n = 8)
[c] (*P* = .072)

Table 11. Prefecal mass (PFM, kg DM) from the chromium one-compartment model, using total collection data, for horses offered orchardgrass/alfalfa hay (OGTC, Diet 1a) and tall fescue/alfalfa hay (TFTC, Diet 2a) in Experiment 1[a]

	Diet 1a	Diet 2a
Horse	OGTC	TFTC
1	3.99	3.38
2	3.89	5.02
3	3.92	4.53
4	4.46	3.07
5	3.44	3.21
6	3.92	2.95
7	3.37	3.68
8	3.58	3.76
Mean ± SE[b]	3.82 ± .12[c]	3.70 ± .25[c]

[a] PFM is prefecal mass or mixing compartment, PFM = FO • TT
[b] Standard error of the mean (n = 8)
[c] (*P* = .702)

Table 12. Prefecal mass (PFM, kg DM) from the chromium one-compartment model, using fecal grab data, for horses offered orchardgrass/alfalfa hay (OGGR, Diet 1b) and tall fescue/alfalfa hay (TFGR, Diet 2b) in Experiment 1[a]

	Diet 1b	Diet 2b
Horse	OGTC	TFTC
1	4.09	3.99
2	3.81	4.13
3	4.06	4.66
4	4.83	3.99
5	3.82	3.66
6	3.64	3.03
7	3.00	3.59
8	4.04	3.94
Mean ± SE[b]	3.91 ± .18[c]	3.90 ± .16[c]

[a] PFM is prefecal mass or mixing compartment, PFM = FO • TT
[b] Standard error of the mean (n = 8)
[c] (*P* = .852)

Table 13. Fecal recovery of chromium (R_{CR}, %) for horses offered orchardgrass/alfalfa hay (Diet 1a) and tall fescue/alfalfa hay (Diet 2a), using total collection fecal samples, in Experiment 1[a]

Horse	Diet 1a	Diet 2a
1	100.8	95.3
2	81.3	122.3
3	71.6	81.5
4	105.0	93.7
5	102.3	113.4
6	119.7	114.1
7	69.7	78.2
8	101.5	120.2
Mean ± SE[b]	94.0 ± 6[c]	102.3 ± 3[c]

[a] Mean of Periods 1 and 2
[b] Standard error of the mean (n = 8)
[c] (*P* = .218)

Table 14. Fecal recovery of chromium (R_{CR}, %) for horses offered orchardgrass/alfalfa hay (Diet 1b) and tall fescue/alfalfa hay (Diet 2b), using fecal grab data, in Experiment 1[a]

Horse	Diet 1b	Diet 2b
1	90.9	85.4
2	87.1	80.5
3	85.4	78.9
4	91.7	74.5
5	94.7	90.5
6	83.3	98.4
7	75.4	74.7
8	90.8	88.8
Mean ± SE[b]	87.4 ± 2.2[c]	83.9 ± 2.9[c]

[a] Mean of Periods 1 and 2
[b] Standard error of the mean (n = 8)
[c] (*P* = .864)

Table 15. Diurnal variation in fecal concentrations (mg/kg, DM) of chromium for individual horse fecal grab samples collected on d 8 of dosing, for horses offered orchardgrass/alfalfa hay (Diet 1) in Experiment 1

	Diurnal Fecal Grab Samples[a]				
Horse	0700	1500	2300	Mean Diurnal	Mixed[b]
1	6991.3	3972.5	3263.8	4742.5	3876.3
2	5127.5	4742.5	4095.0	4655.1	3990.0
3	4803.8	6378.8	5740.0	5640.8	4786.3
4	3395.0	2861.3	2852.5	3036.2	2633.8
5	2887.5	2222.5	3237.5	2782.5	2467.5
6	3307.5	4943.8	5853.8	4701.6	3823.8
7	5512.5	3920.0	4147.5	4526.6	3928.8
8	2852.5	2310.0	3351.3	2838.1	2765.0
Mean ± SE[c]	4359 ± 526[d]	3918 ± 506[d]	4067 ± 407[d]	4115 ± 380[e]	3533 ± 288[e]

[a] Fecal grab samples collected at 0700, 1500 and 2300
[b] Fecal grab samples collected at 0700, 1500 and 2300, and combined
[c] Standard error of the mean (n = 8)
[d] Coefficient of variation (5.44 %)
[e] (*P* = .0009)

Table 16. Diurnal variation in fecal concentrations (mg/kg, DM) of chromium for individual horse fecal grab samples collected on d 8 of dosing, for horses offered tall fescue/alfalfa hay (Diet 2) in Experiment 1

	Diurnal Fecal Grab Samples[a]				
Horse	0700	1500	2300	Mean Diurnal	Mixed[b]
1	4725.0	5355.0	3631.3	4570.4	3675.0
2	4331.3	4287.5	2257.5	3625.4	2476.3
3	5547.5	5530.0	4182.5	5086.6	4322.5
4	3788.8	2450.0	2730.0	2989.5	2367.5
5	2625.0	2432.5	2992.5	2683.3	2633.8
6	4665.0	3001.3	4235.0	3967.1	3745.0
7	4952.5	4873.8	4322.5	4716.3	4217.5
8	3316.3	2747.5	3290.0	3118.1	2677.5
Mean ± SE[c]	4243 ± 335[d]	3834 ± 467[d]	3455 ± 271[d]	3844 ± 313[e]	3264 ± 286[e]

[a] Fecal grab samples collected at 0700, 1500 and 2300
[b] Fecal grab samples collected at 0700, 1500 and 2300, and combined
[c] Standard error of the mean (n = 8)
[d] Coefficient of variation (10.25 %)
[e] (P = .0025)

Table 17. Partial DMD (D_S, %) of fat-and-fiber, and sugar-and-starch supplements offered to horses in Experiment 2

Horse	Fat-and Fiber	Sugar-and-Starch
1	72.9	66.3
2	67.3	68.3
3	68.8	70.6
4	68.2	78.6
5	63.1	64.9
6	79.6	63.8
7	68.1	68.0
8	79.2	77.0
Mean ± SE[a]	70.9 ± 2.1[b]	69.7 ± 1.9[b]

[a] Standard error of the mean (n = 8)
[b] (P = .703)

Table 18. Fecal recovery of yttrium (R_Y, %) for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3) and orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4) in Experiment 2[a]

Horse	Diet 3	Diet 4
1	120.9	93.5
2	97.6	118.8
3	102.7	90.2
4	98.4	120.2
5	97.2	125.1
6	100.1	98.8
7	110.3	102.7
8	95.0	129.9
Mean ± SE[b]	102.8 ± 3.1[c]	109.9 ± 5.4[c]

[a] Mean of Periods 1 and 2
[b] Standard error of the mean (n = 8)
[c] (P = .115)

Table 19. Total collection DMD (D_{TC}, %) and estimated DMD (D_E, %) for yttrium, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3), and orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4) in Experiment 2.

	Diet 3		Diet 4	
Horse	D_{TC}	D_E	D_{TC}	D_E
1	64.97	73.43	62.86	63.57
2	66.10	66.14	66.06	73.79
3	62.83	66.80	59.72	58.05
4	58.58	60.60	68.66	76.08
5	60.78	62.97	61.24	71.58
6	67.46	70.17	62.14	64.85
7	63.32	69.48	63.69	67.57
8	63.75	64.98	66.53	76.38
Mean ± SE[a]	63.4 ± 1.0[b]	66.8 ± 1.4[b]	63.8 ± 1.0[c]	68.9 ± 2.3[c]

[a] Standard error of the mean (n = 8)
[b] (P = .012); [c] (P = .005)

Table 20. Fecal recovery of ytterbium (R_{YB1}, %) for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Yb) and orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Yb) in Experiment 2[a, b]

Horse	Diet 3Yb	Diet 4Yb
1	49.6	53.2
2	49.3	48.2
3	51.8	58.7
4	49.5	36.4
5	41.9	44.4
6	47.1	49.3
7	44.0	44.3
8	41.8	37.6
Mean ± SE[c]	46.9 ± 1.4[d]	46.5 ± 2.6[d]

[a] Mean of Periods 1 and 2
[b] Composite fecal samples of d 5 to d 8 of dosing
[c] Standard error of the mean (n = 8)
[d] (*P* = .871)

Table 21. Total collection DMD (D_{TC}, %) and estimated DMD (D_E, %) for ytterbium, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3), and orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4) in Experiment 2

Horse	Diet 3		Diet 4	
	D_{TC}	D_E	D_{TC}	D_E
1	64.97	29.37	62.86	30.20
2	66.10	31.18	66.06	29.53
3	62.83	28.25	59.72	31.36
4	58.58	16.40	68.66	13.98
5	60.78	6.28	61.24	12.76
6	67.46	30.96	62.14	23.22
7	63.32	16.72	63.69	18.11
8	63.75	13.26	66.53	11.05
Mean ± SE[a]	63.4 ± 1.0[b]	21.5 ± 3.3[b]	63.8 ± 1.0[c]	21.3 ± 2.9[c]

[a] Standard error of the mean (n = 8)
[b] ($P < .001$); [c] ($P < .001$)

Table 22. Estimates of fit of pooled fecal chromium or ytterbium concentration data to a one-compartment model, model parameters, and calculated variables in horses offered orchardgrass/alfalfa hay and fat-and-fiber (FF) supplement (Diet 3Cr and 3Yb), or sugar-and-starch (SS) supplement (Diet 4Cr and 4Yb), in Experiment 2[a]

Estimate	Diet 3Cr OGFF	Diet 4Cr OGSS	Diet 3Yb OGFF	Diet 4Yb OGSS
R^2	.788	.872	.895	.917
F	26.03	81.48	110.25	143.23
P-value	.00140	.00000	.00000	.00000
Cr_a, mg/kg[b]	5.140	5.537	.145	.151
SE[c]	.633	.510	.010	.010
t-statistic	8.113	10.847	13.777	14.463
P-value	.00008	.00001	.00000	.00000
k, d^{-1d}	1.060	.816	.825	.731
SE	.471	.370	.320	.321
t-statistic	1.999	3.299	3.781	4.254
P-value	.08571	.01313	.00688	.00377
TT, h[e]	22.63	29.38	29.08	32.81
Delay, h[f]	6.792	6.648	5.136	5.520
FO_{CA}, kg/d[g]	3.754	3.485	4.614	4.424
PFM, kg[h]	3.540	4.266	5.592	6.049

[a] Pooled data = mean of eight horses; [b] Cr_a is the asymptotic value of fecal Cr concentration
[c] Standard error of the mean (n = 9); [d] k is the rate constant; [e] TT is turnover time of the prefecal mass, TT = -1/k
[f] Delay is the time between the administration of Cr and its entry into the prefecal mass
[g] FO_{CA}, is the fecal output, FO_{CA} = dose of Cr (Yb)/Cr_a; [h] PFM is prefecal mass or mixing compartment, PFM = FO_{CA} • TT.

Table 23.

Fits of data to a one-compartment model of fecal kinetics without a delay, the administration curve, the post-administration curve, and the total curve with a delay between administration of an oral dose of Cr or Yb and its entry into the compartment, in horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement using Cr (Diet 3Cr), or Yb (Diet 3Yb), or orchardgrass/alfalfa hay and sugar-and-starch supplement using Cr (Diet 4Cr), or Yb (Diet 4Yb), in Experiment 2[a]

	No delay			Administration curve			Post-Administration Curve			Total Curve		
Diet	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value
Diet 3Cr OGFF[b]	.689	15.51	.0056	.788	26.04	.0014	.755	9.24	.0559	.631	20.49	.0007
Diet 4Cr OGSS[c]	.775	24.16	.0017	.845	38.04	.0005	.892	24.78	.0156	.872	81.48	.0000
Diet 3Yb OGFF[d]	.851	40.08	.0004	.895	59.83	.0001	.943	66.57	.0012	.917	143.24	.0000
Diet 4Yb OGSS[e]	.823	32.51	.0007	.873	48.00	.0002	.919	45.20	.0026	.895	110.25	.0000

[a] Pooled data for eight horses
[b] Delays were calculated for each model: [b] (6.792 h); [c] (6.648 h); [d] (5.520 h); [e] (5.136 h)

Table 24.

Fits of data to a one-compartment model of fecal kinetics without a delay, the administration curve with a delay, the post-administration curve, and the total curve with a delay between administration of an oral dose of Cr and its entry into the compartment, in horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement in Experiment 2[a, b, c]

	No Delay			Administration Curve			Post-Administration Curve			Total Curve		
Horse	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value
1	.781	25.03	.0016	.855	70.79	.0001	.869	19.93	.0209	.853	40.71	.0000
2	.699	16.26	.0050	.798	27.63	.0012	.912	20.80	.0449	.798	43.47	.0000
3	.844	37.95	.0005	.894	59.01	.0001	.608	4.65	.1201	.839	62.38	.0000
4	.604	10.69	.0137	.716	17.66	.0040	.809	8.49	.1003	.797	47.10	.0000
5	.691	15.67	.0055	.760	22.19	.0022	.561	2.56	.2509	.774	41.15	.0001
6	.907	67.95	.0001	.920	80.46	.0000	.671	4.09	.1806	.912	124.18	.0000
7	.789	26.15	.0014	.857	42.11	.0003	.808	12.62	.0380	.874	83.07	.0000
8	.664	13.81	.0075	.770	23.45	.0019	.552	2.47	.2567	.688	26.41	.0003

[a] Individual horse data
[b] Delay from mean OGSS model (6.648 h)
[c] Model fitted using the chromium external marker

Table 25.

Fits of data to a one-compartment model of fecal kinetics without a delay, the administration curve with a delay, the post-administration curve, and the total curve with a delay between administration of an oral dose of Yb and its entry into the compartment, in horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement in Experiment 2[a, b, c]

	No Delay			Administration Curve			Post-Administration Curve			Total Curve		
Horse	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value
1	.704	16.65	.0047	.799	27.77	.0012	.819	18.04	.0132	.802	52.65	.0000
2	.730	18.91	.0034	.817	31.29	.0008	.938	60.75	.0015	.871	87.80	.0000
3	.828	33.63	.0007	.877	49.99	.0002	.621	4.92	.0909	.816	53.34	.0000
4	.642	12.55	.0094	.753	21.37	.0024	.939	61.85	.0014	.819	58.70	.0000
5	.773	23.79	.0018	.823	32.55	.0007	.771	13.47	.0214	.848	72.32	.0000
6	.966	201.41	.0000	.880	51.47	.0002	.841	21.09	.0101	.883	97.93	.0000
7	.909	69.86	.0001	.858	42.20	.0003	.845	21.79	.0095	.857	77.76	.0000
8	.636	12.21	.0101	.743	20.19	.0028	.927	50.91	.0020	.811	55.90	.0000

[a] Individual horse data
[b] Delay from mean OGSS model (5.136 h)
[c] Model fitted using the ytterbium external marker

Table 26. Estimates of fit of individual fecal chromium concentration data to a one-compartment model, model parameters, and calculated variables in horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Cr) in Experiment 2

Horse	Cr_a, mg/kg[a]	FO_{CA}, kg/d[b]	TT, h[c]	PFM, kg[d]	R^2	*F*	*P*-value
1	4.764	4.050	22.21	3.748	.742	20.09	.00286
2	4.989	3.868	33.36	5.376	.923	84.44	.00004
3	5.373	3.591	20.76	3.107	.750	21.02	.00253
4	4.838	3.988	21.45	3.565	.789	26.12	.00138
5	4.670	4.132	26.19	4.509	.878	79.06	.00005
6	5.795	3.330	19.05	2.643	.715	17.56	.00409
7	5.898	3.271	25.80	3.517	.702	16.52	.00479
8	3.852	5.009	26.60	5.552	.824	32.82	.00071

[a] Cr_a is the asymptotic value of fecal Cr concentration
[b] FO_{CA} is the fecal output, FO_{CA} = dose of Cr/Cr_a
[c] TT is turnover time of the prefecal mass, TT = -1/k
[d] PFM is prefecal mass or mixing compartment, PFM = FO_{CA} • TT

Table 27. Estimates of fit of individual fecal chromium concentration data to a one-compartment model, model parameters, and calculated variables in horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Cr) in Experiment 2

Horse	Cr_a, mg/kg[a]	FO_{CA}, kg/d[b]	TT, h[c]	PFM, kg[d]	R^2	*F*	*P*-value
1	5.577	3.460	25.25	3.641	.853	40.71	.00037
2	5.083	3.796	23.77	3.760	.798	43.47	.00031
3	5.508	3.503	32.74	4.779	.894	59.01	.00012
4	5.327	3.622	27.78	4.193	.716	17.66	.00402
5	5.097	3.786	20.43	3.223	.760	22.19	.00218
6	5.590	3.452	28.71	4.129	.920	80.46	.00004
7	5.014	3.848	32.35	5.188	.874	83.07	.00004
8	5.328	3.621	25.50	3.849	.770	23.45	.00187

[a] Cr_a is the asymptotic value of fecal Cr concentration
[b] FO_{CA} is the fecal output, FO_{CA} = dose of Cr/Cr_a
[c] TT is turnover time of the prefecal mass, TT = -1/k
[d] PFM is prefecal mass or mixing compartment, PFM = FO_{CA} • TT

Table 28. Total collection fecal output (FO_{TC}, kg/d DM), and estimates of fecal output (FO_{CA}, kg/d DM) from the one-compartment model, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (OGFF, Diet 3) or sugar-and-starch supplement (OGSS, Diet 4), with chromium as the external marker, in Experiment 2[a]

	Diet 3Cr		Diet 4Cr	
	OGFF	OGFF	OGSS	OGSS
Horse	FO_{TC}	FO_{CA}	FO_{TC}	FO_{CA}
1	2.78	4.05	2.91	3.46
2	2.66	3.86	2.86	3.79
3	2.74	3.59	3.04	3.50
4	2.71	3.98	2.12	3.62
5	3.05	4.13	2.82	3.78
6	2.31	3.33	2.50	3.45
7	2.30	3.27	2.40	3.84
8	2.54	5.01	2.26	3.62
Mean ± SE[b]	2.64 ± .08[c]	3.90 ± .19[c,e]	2.61 ± .12[d]	3.63 ± .05[d,e]

[a] FO_{CA} = dose of Cr/C_a (asymptote)
[b] Standard error of the mean (n = 8)
[c] (P = .004); [d] (P = .028); [e] (P = .137)

Table 29. Total collection fecal output (FO_{TC}, kg/d DM), and estimates of fecal output (FO_{CA}, kg/d DM) from the one-compartment model, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (OGFF, Diet 3) or sugar-and-starch supplement (OGSS, Diet 4), with ytterbium as the external marker, in Experiment 2[a]

	Diet 3Yb		Diet 4Yb	
	OGFF	OGFF	OGSS	OGSS
Horse	FO_{TC}	FO_{CA}	FO_{TC}	FO_{CA}
1	2.78	4.51	2.91	4.43
2	2.66	4.44	2.86	4.66
3	2.74	4.31	3.04	4.25
4	2.71	4.65	2.12	4.46
5	3.05	4.95	2.82	4.61
6	2.31	4.07	2.50	4.09
7	2.30	4.61	2.40	4.40
8	2.54	5.12	2.26	4.38
Mean ± SE[b]	2.64 ± .08[c]	4.58 ± .11[c,e]	2.61 ± .12[d]	4.41 ± .06[d,e]

[a] FO_{CA} = dose of Yb/C_a (asymptote)
[b] Standard error of the mean (n = 8)
[c] (P = .0001); [d] (P = .0002); [e] (P = .084)

Table 30. Turnover times (TT, h) from the chromium one-compartment model, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (OGFF, Diet 3Cr), and orchardgrass/alfalfa hay and sugar-and-starch supplement (OGSS, Diet 4Cr) in Experiment 2[a]

	Diet 3Cr	Diet 4Cr
Horse	OGFF	OGSS
1	22.21	25.25
2	33.36	23.77
3	20.76	32.74
4	21.45	27.78
5	26.19	20.43
6	19.05	28.71
7	25.80	32.35
8	26.60	25.50
Mean ± SE[b]	24.42 ± 1.61[c]	27.06 ± 1.41[c]

[a] TT is turnover time of the prefecal mass, TT = -1/k
[b] Standard error of the mean (n = 8)
[c] (*P* = .356)

Table 31. Turnover times (TT, h) from the ytterbium one-compartment model, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (OGFF, Diet 3Yb), and orchardgrass/alfalfa hay and sugar-and-starch supplement (OGSS, Diet 4Yb) in Experiment 2[a]

	Diet 3Yb	Diet 4Yb
Horse	OGFF	OGSS
1	26.34	28.45
2	29.75	23.84
3	27.05	24.47
4	24.50	23.51
5	24.11	25.75
6	23.77	26.04
7	25.11	32.61
8	26.18	23.23
Mean ± SE[b]	25.85 ± .69[c]	25.98 ± 1.12[c]

[a] TT is turnover time of the prefecal mass, TT = -1/k
[b] Standard error of the mean (n = 8)
[c] (*P* = .928)

Table 32. Prefecal mass (PFM, kg DM) from the chromium one-compartment model, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (OGFF, Diet 3Cr), and orchardgrass/alfalfa hay and sugar-and-starch supplement (OGSS, Diet 4Cr) in Experiment 2[a]

	Diet 3Cr	Diet 4Cr
Horse	OGFF	OGSS
1	3.74	3.64
2	5.37	3.76
3	3.10	4.77
4	3.56	4.19
5	4.50	3.22
6	2.64	4.12
7	3.51	5.18
8	5.55	3.84
Mean ± SE[b]	4.00 ± .37[c]	4.09 ± .22[c]

[a] PFM is prefecal mass or mixing compartment, PFM = FO • TT
[b] Standard error of the mean (n = 8)
[c] (*P* = .864)

Table 33. Prefecal mass (PFM, kg DM) from the ytterbium one-compartment model, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (OGFF, Diet 3Yb), and orchardgrass/alfalfa hay and sugar-and-starch supplement (OGSS, Diet 4Yb) in Experiment 2[a]

	Diet 3Yb	Diet 4Yb
Horse	OGFF	OGSS
1	4.95	5.25
2	5.51	4.63
3	4.86	4.33
4	4.75	4.37
5	4.98	4.95
6	4.03	4.44
7	4.82	5.98
8	5.59	4.24
Mean ± SE[b]	4.93 ± .17[c]	4.77 ± .21[c]

[a] PFM is prefecal mass or mixing compartment, PFM = FO • TT
[b] Standard error of the mean (n = 8)
[c] (*P* = .581)

Table 34. Fecal recovery of chromium (R_{CR}, %) for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Cr), and orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Cr) in Experiment 2[a]

Horse	Diet 3Cr	Diet 4Cr
1	68.6	84.1
2	68.7	75.3
3	76.2	86.7
4	67.9	58.5
5	73.8	74.5
6	69.4	72.4
7	70.3	62.4
8	50.7	62.4
Mean ± SE[b]	68.2 ± 2.7[c]	72.1 ± 3.2[c]

[a] Mean of Periods 1 and 2
[b] Standard error of the mean (n = 8)
[c] (P = .570)

Table 35. Fecal recovery of ytterbium (R_{YB2}, %) for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Yb) and offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Yb) in Experiment 2[a, b]

Horse	Diet 3Yb	Diet 4Yb
1	61.5	65.6
2	59.8	61.3
3	63.5	71.5
4	58.2	48.5
5	61.5	61.1
6	56.7	61.1
7	49.9	54.5
8	50.2	51.5
Mean ± SE[b]	58.6 ± 1.8[c]	59.3 ± 2.7[c]

[a] Mean of Periods 1 and 2
[b] Fecal samples of d 1 to d 8 of dosing
[c] Standard error of the mean (n = 8)
[d] (P = .883)

Table 36. Fecal dry matter (DM, % wet weight) of horses offered orchardgrass/alfalfa hay (Diet 1), tall fescue/alfalfa hay (Diet 2), orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3), and orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4), in Experiment 1 and 2

	DM %		DM %	
	Diet 1	Diet 2	Diet 3	Diet 4
Horse				
1	21.02	21.51	27.20	27.92
2	22.42	20.29	25.87	29.55
3	20.58	21.03	27.12	28.19
4	21.02	18.52	26.11	27.35
5	21.51	19.92	24.20	25.97
6	20.29	21.52	22.54	25.61
7	21.03	20.24	25.46	26.16
8	18.52	20.32	25.02	27.03
Mean ± SE[a]	20.79 ± .39[b,d,e]	20.42 ± .34[b]	25.44 ± .54[c,d]	27.22 ± .46[c,e]

[a] Standard error of the mean
[b] (P = .524); [c] (P = .002); [d] (P = .0001); [e] (P = .001)

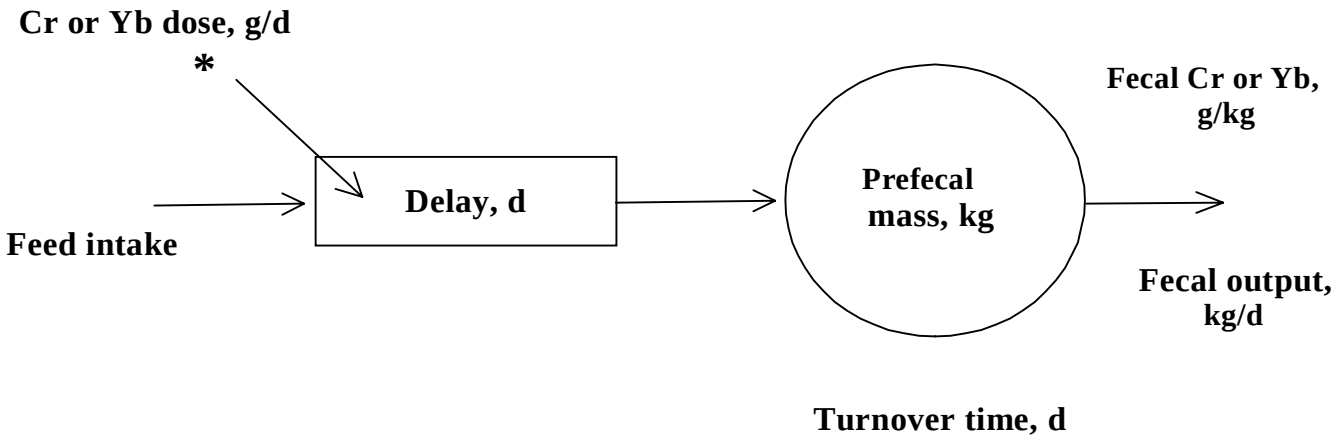

Figure 1. A one-compartment model of the prefecal mass (PFM, kg), or mixing compartment, sampled by feces, and fecal output (FO, kg/d), with a delay (*d*, d) between oral administration of Cr or Yb dose (*) and the entry of this Cr or Yb into the PFM. The fecal Cr or Yb concentrations (mg/kg DM) at time *t* (d), C_t, rise to an asymptotic value, C_a, and can be used to determine a single rate constant, k (d^{-1}): $C_t = C_a - C_a.e^{-k(t - d)}$.

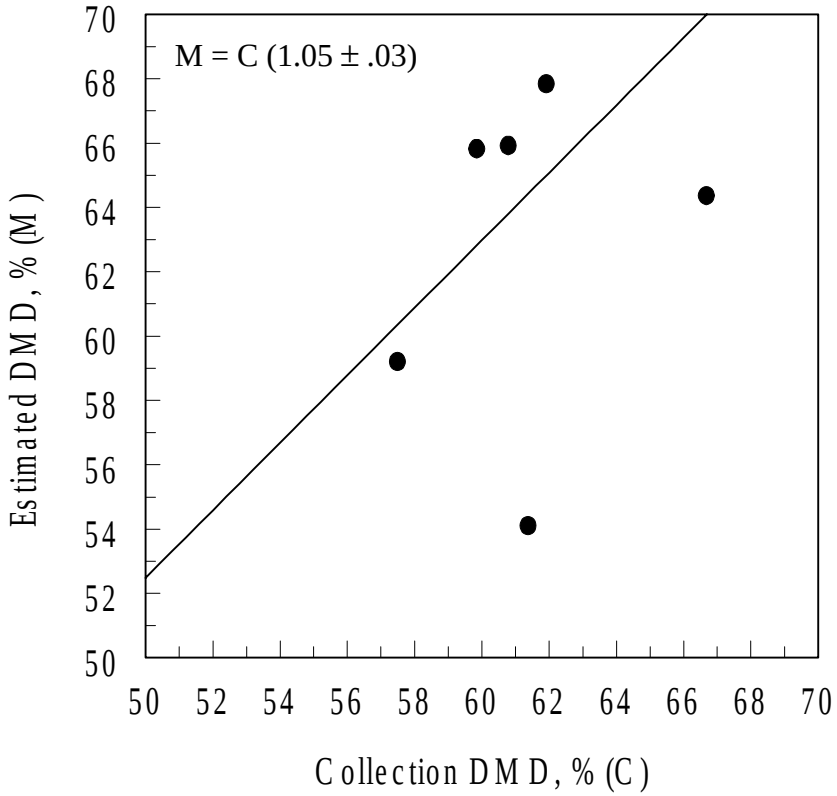

Figure 2. Total collection (C) of DMD and corresponding marker estimates (M) of DMD predicted by yttrium marker dilution, were correlated for horses offered orchardgrass/alfalfa hay (Diet 1) in Experiment 1.

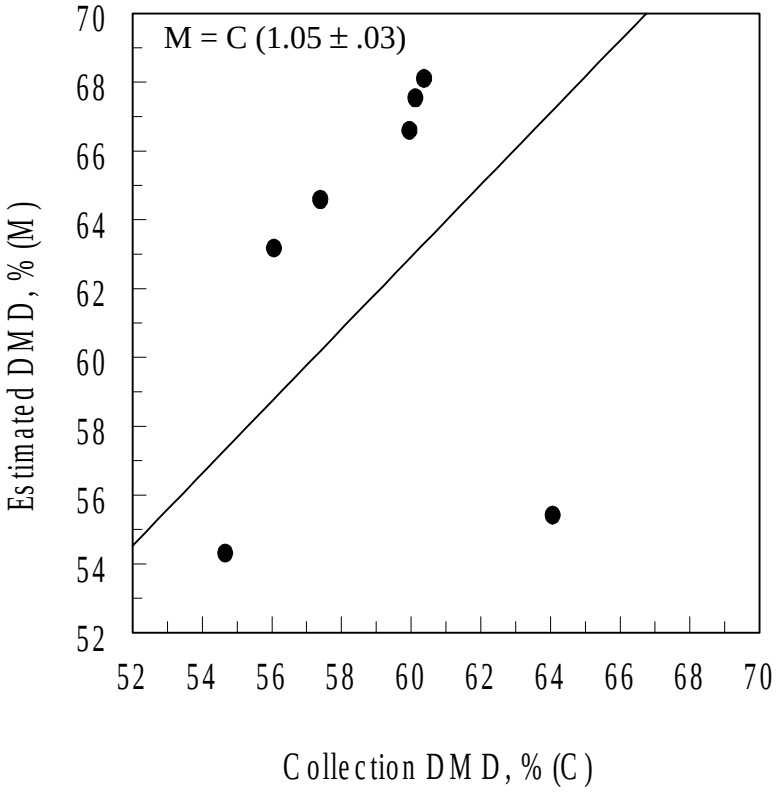

Figure 3. Total collection (C) of DMD and corresponding marker estimates (M) of DMD predicted by yttrium marker dilution were correlated, for horses offered tall fescue/alfalfa hay (Diet 2) in Experiment 1.

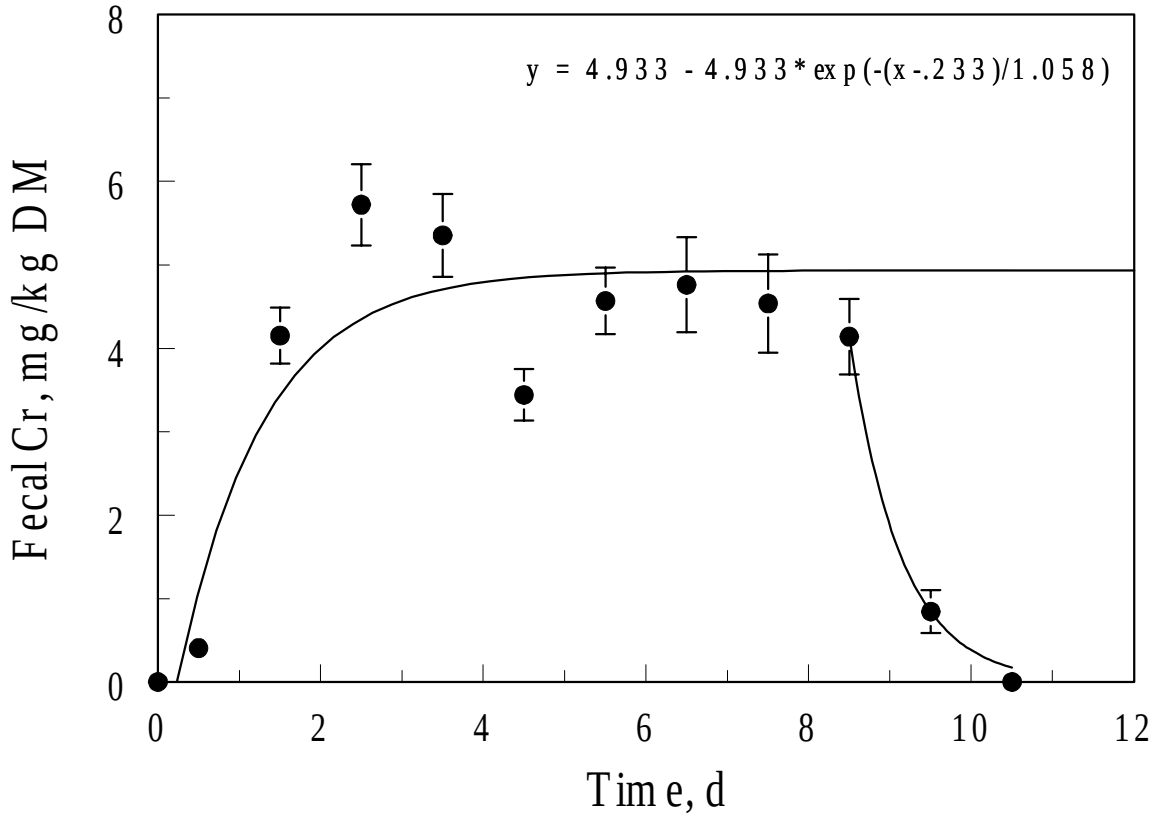

Figure 4.

Mean daily fecal concentrations of Cr, C_t, for horses offered orchardgrass/alfalfa hay (Diet 1a) are plotted against time, and the data are fit to a one-compartment model using the total collection data.

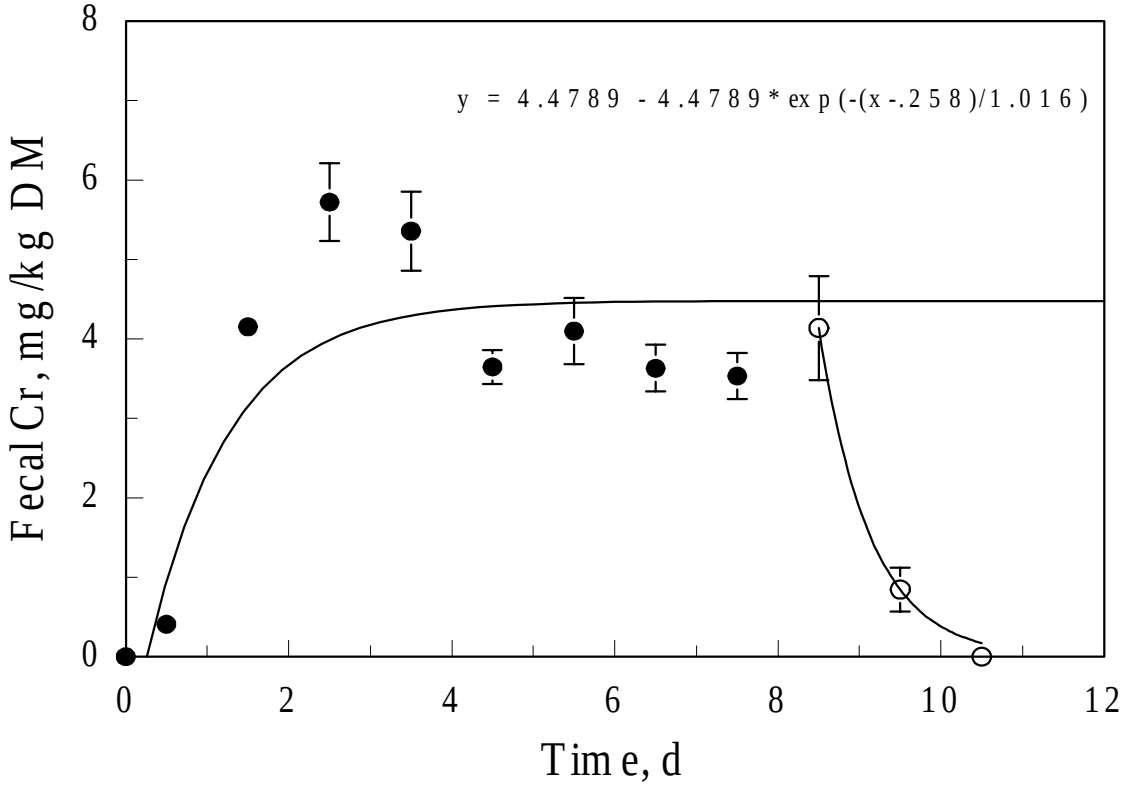

Figure 5.

Mean daily fecal concentrations of Cr, C_t, for horses offered orchardgrass/alfalfa hay (Diet 1b) are plotted against time, and the data are fit to a one-compartment model using the fecal grab data.
Open circles represent the post-administration curve which was not used in the model

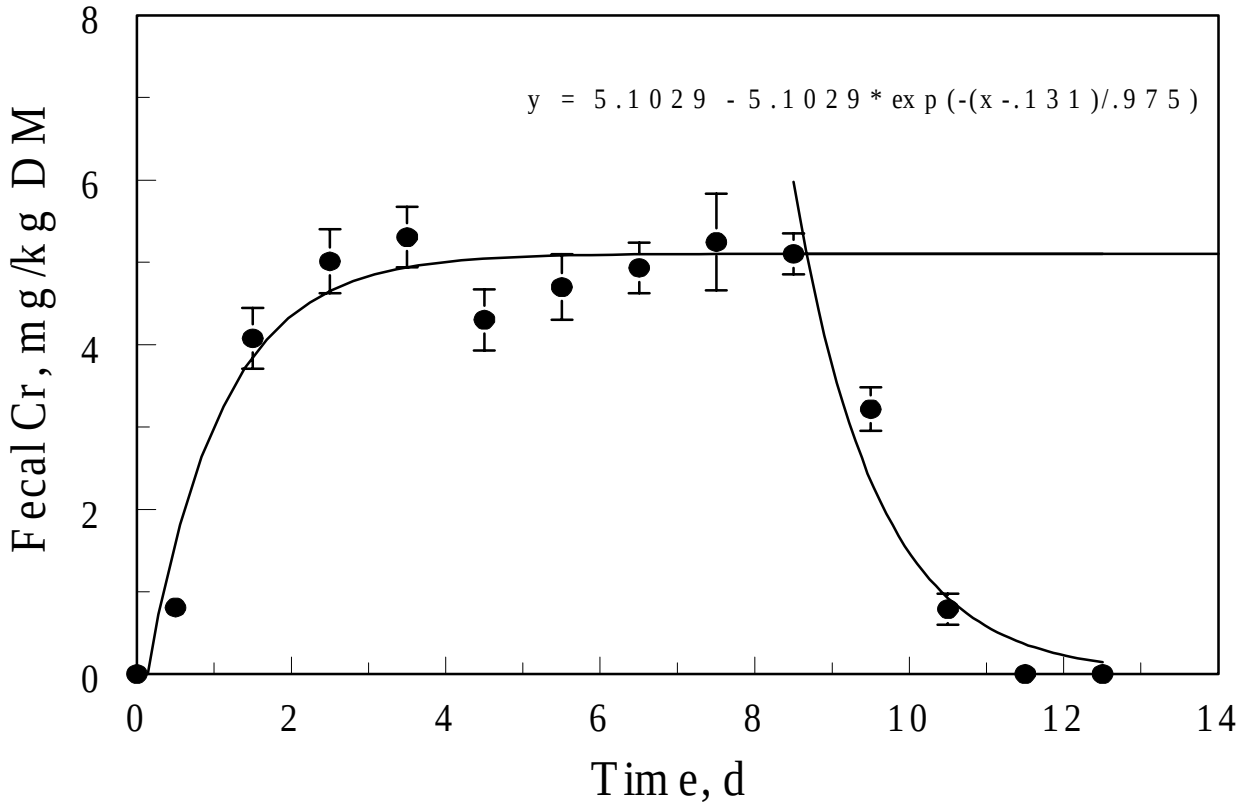

Figure 6.

Mean daily fecal concentrations of Cr, C_t, for horses offered tall fescue/alfalfa hay (Diet 2a) are plotted against time, and the data are fit to a one-compartment model using the total collection data.

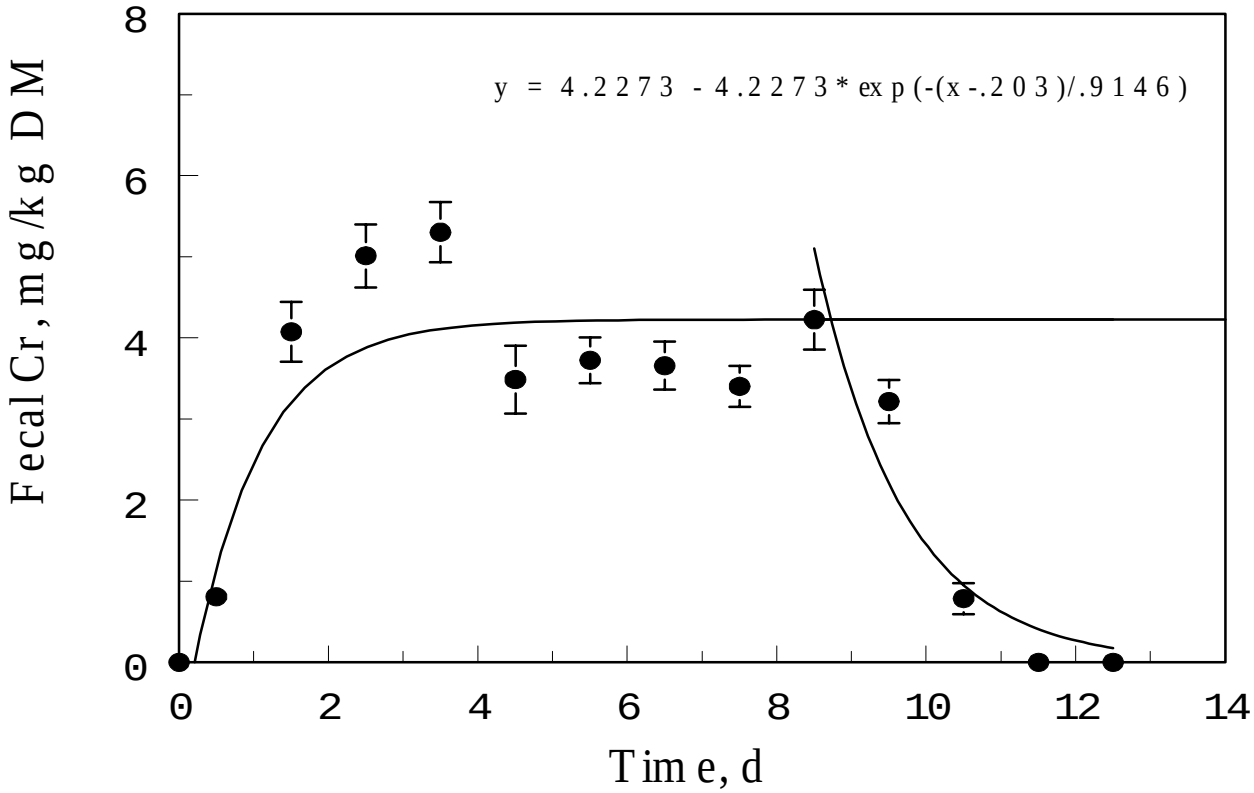

Figure 7.

Mean daily fecal concentrations of Cr, C_t, for horses offered tall fescue/alfalfa hay (Diet 2b) are plotted against time, and the data are fit to a one-compartment model using the fecal grab data.

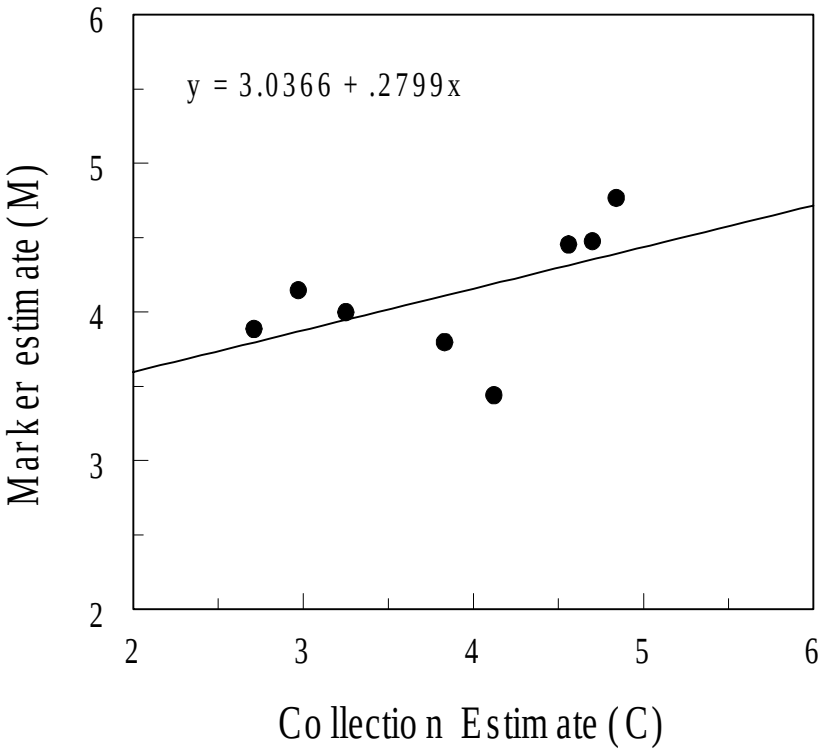

Figure 8. Linear relationship between total collection (C) fecal output and corresponding marker estimates (M) using the total collection Cr data, for horses offered orchardgrass/alfalfa hay (Diet 1a) in Experiment 1.

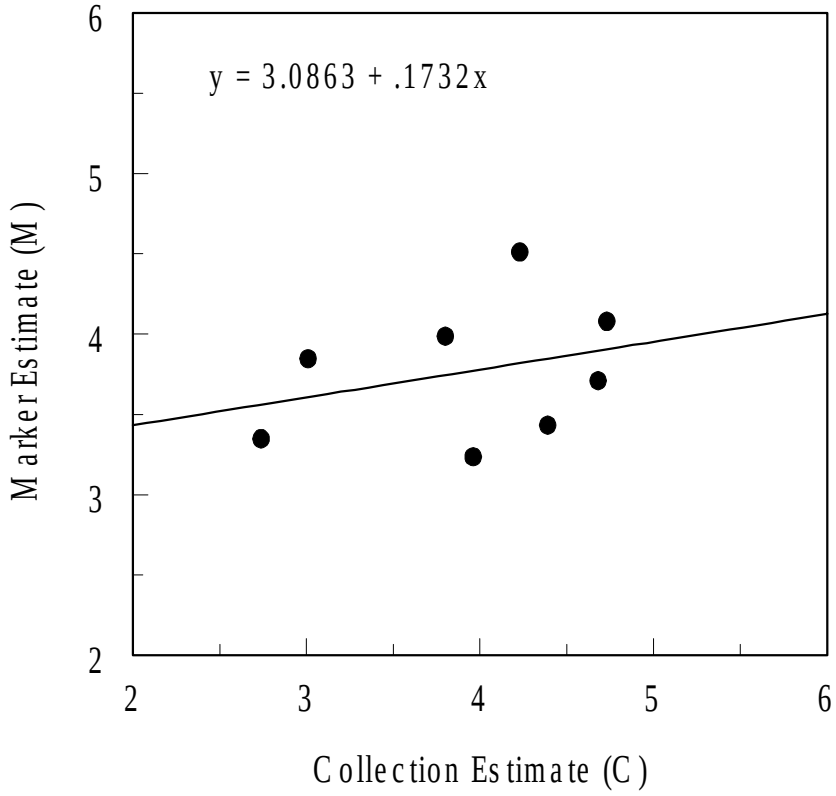

Figure 9. Linear relationship between total collection (C) fecal output and corresponding marker estimates (M) using the total collection Cr data, for horses offered tall fescue/alfalfa hay (Diet 2a) in Experiment 1

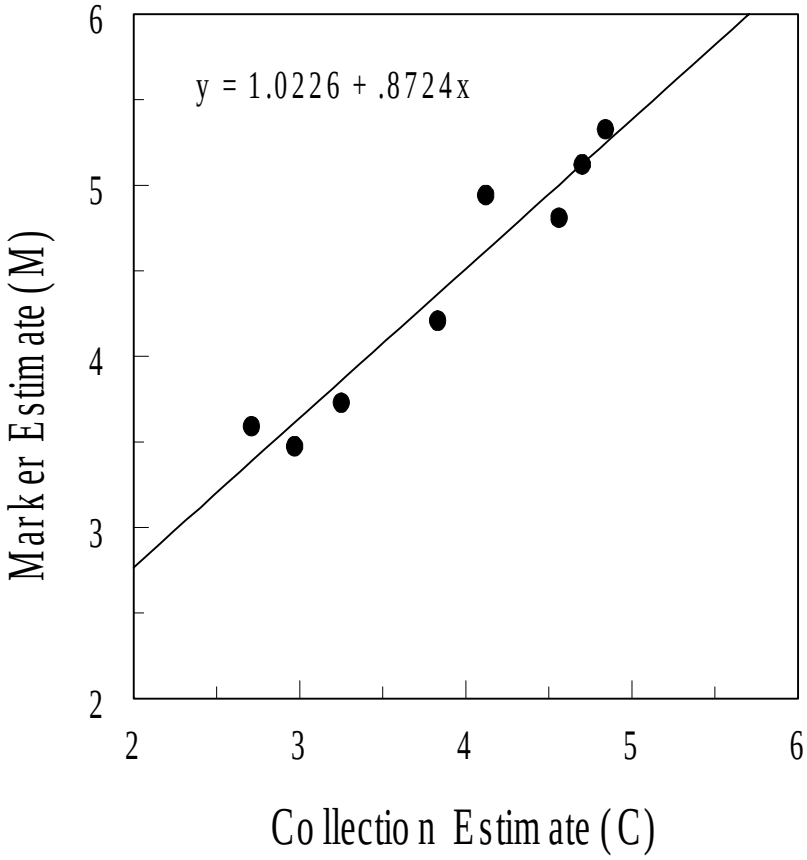

Figure 10. Linear relationship between total collection (C) fecal output and corresponding marker estimates (M) by chromium marker dilution and using the fecal grab data, for horses offered orchardgrass/alfalfa hay (Diet 1b) in Experiment 1.

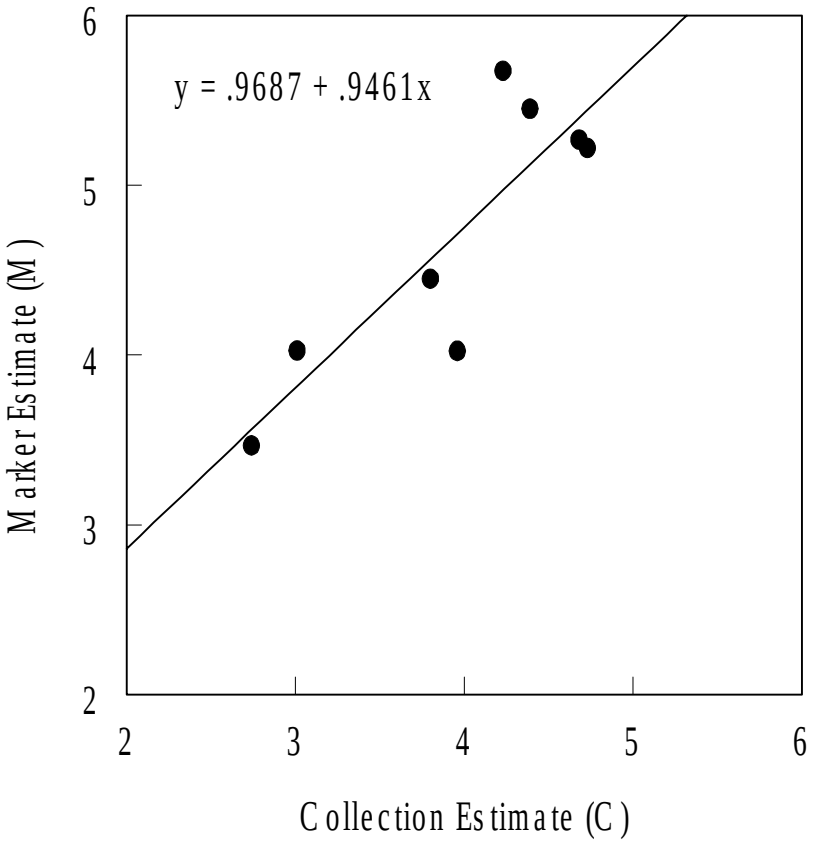

Figure 11. Linear relationship between total collection (C) fecal output and corresponding marker estimates (M) by chromium marker dilution and using the fecal grab data, for horses offered tall fescue/alfalfa hay (Diet 2b) in Experiment 1.

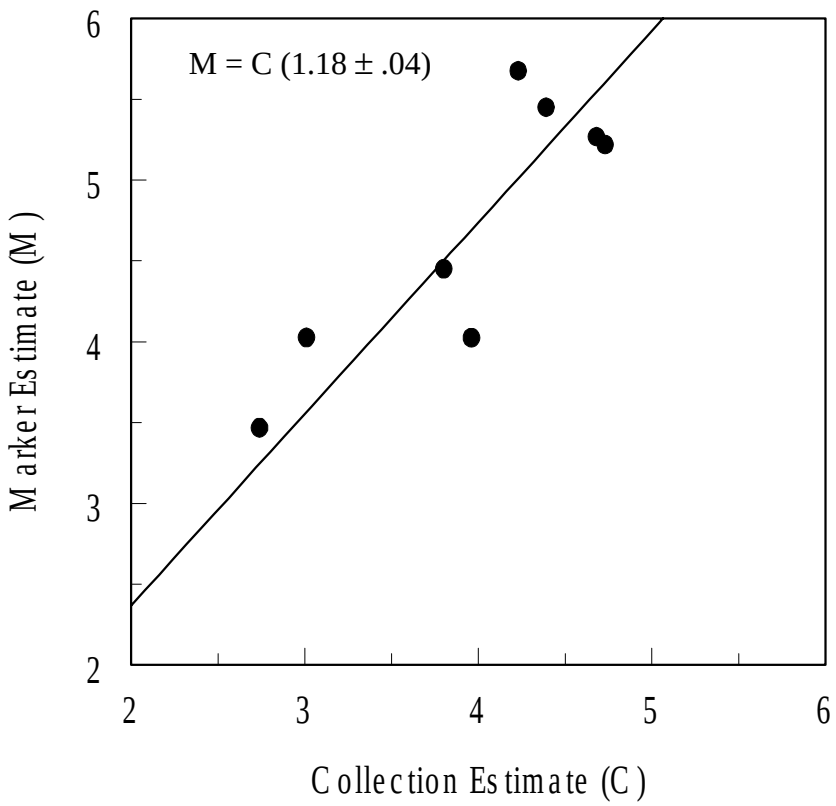

Figure 12. Total collection (C) fecal output and corresponding marker estimates (M), predicted by chromium marker dilution and using the fecal grab data, were correlated for horses offered tall fescue/alfalfa hay (Diet 2b) in Experiment 1

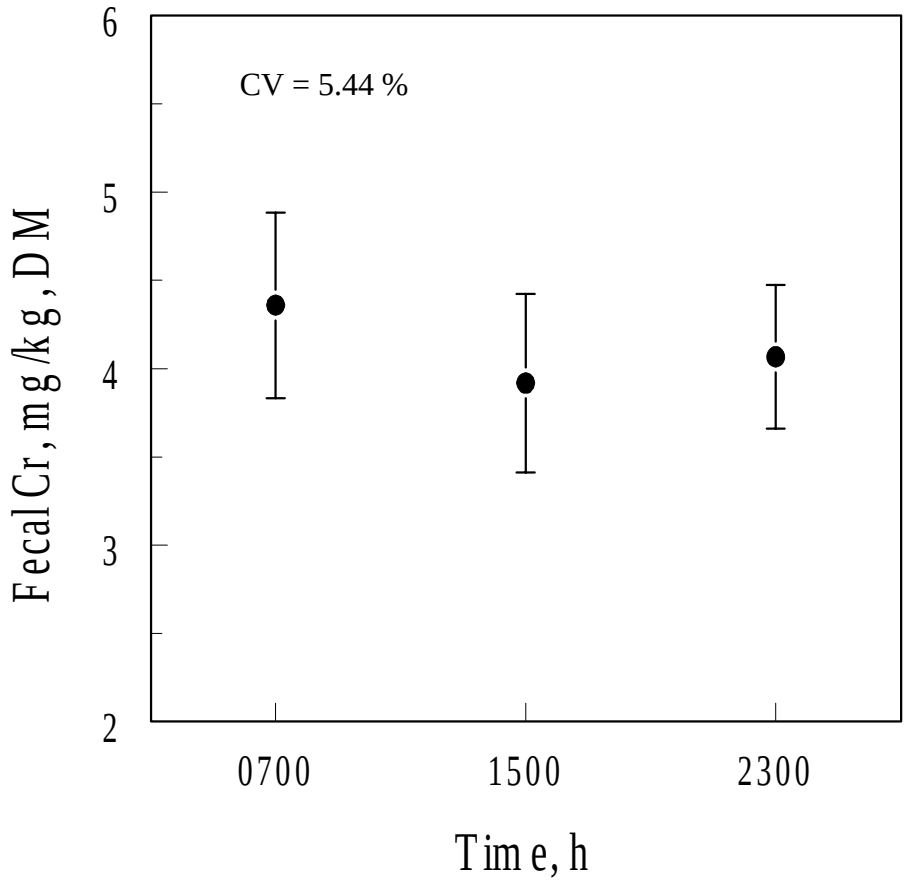

Figure 13. Diurnal variation in fecal concentration (mg/kg, DM) of chromium for individual horse fecal grab samples collected on d 8 of dosing, for horses offered orchardgrass/alfalfa hay (Diet 1) in Experiment 1.

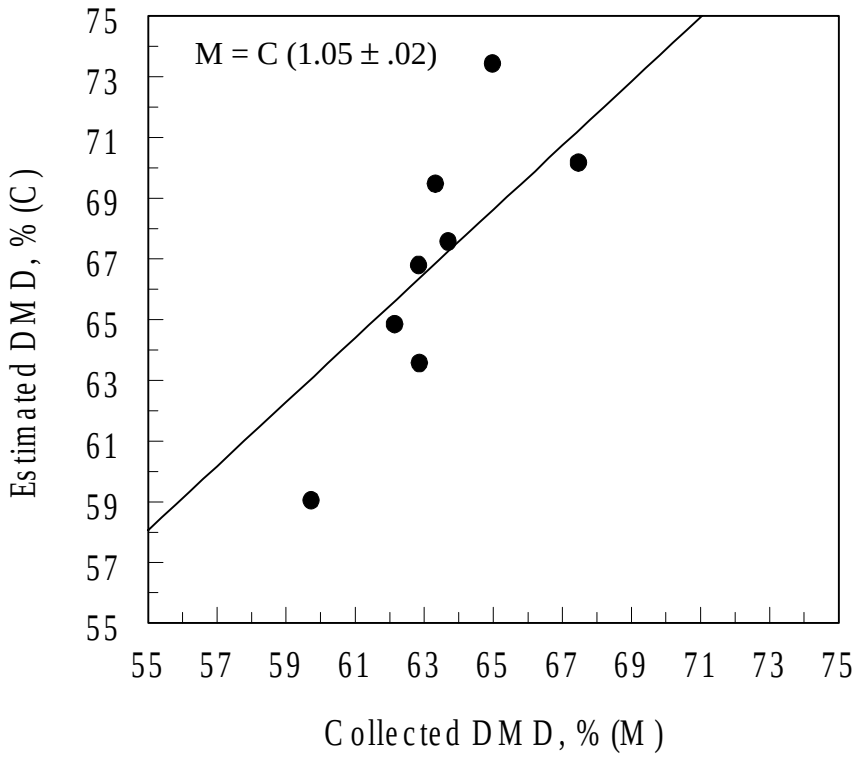

Figure 14. Total collection (C) of DMD and corresponding marker estimates (M) of DMD, predicted by yttrium marker dilution were correlated for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3) in Experiment 2.

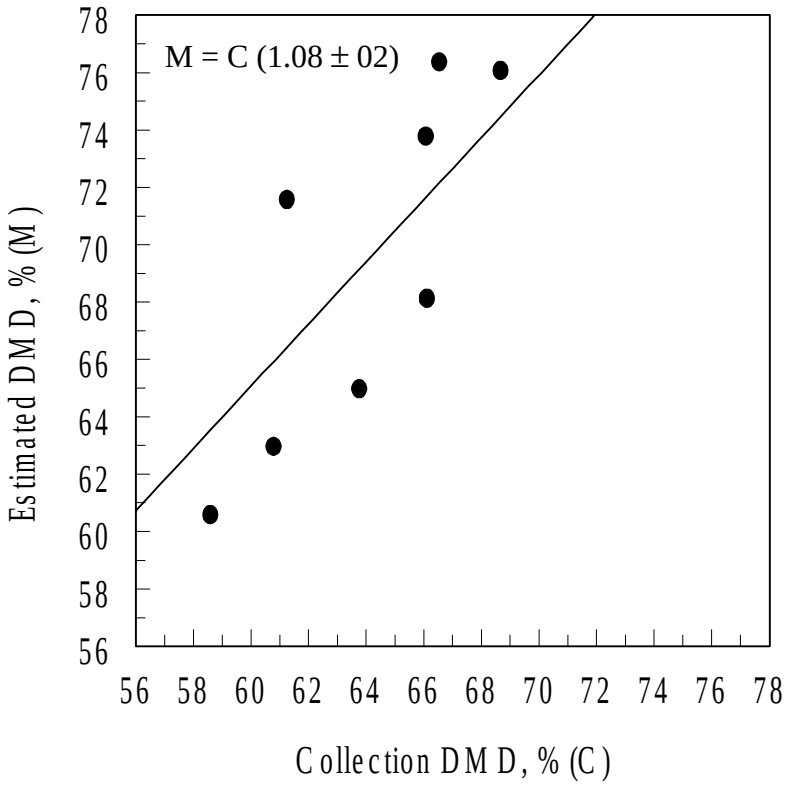

Figure 15. Total collection (C) of DMD and corresponding marker estimates (M) of DMD, predicted by yttrium marker dilution were correlated for horses offered orchardgrass/alfalfa hay and sugar-and-starch (Diet 4) in Experiment 2.

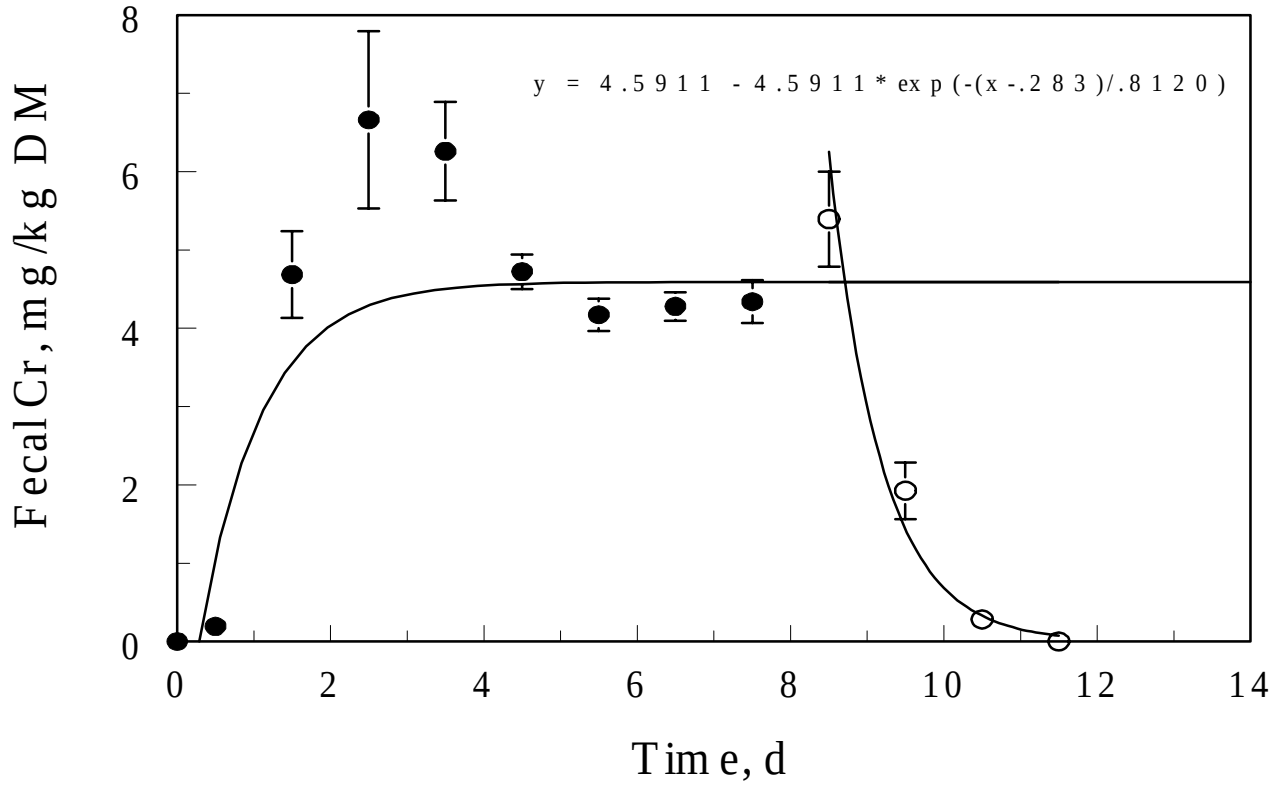

Figure 16.

Mean daily fecal concentrations of Cr, C_t, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Cr) are plotted against time, and the data are fit to a one-compartment model.
Open circles represent the post-administration curve which was not used in the model

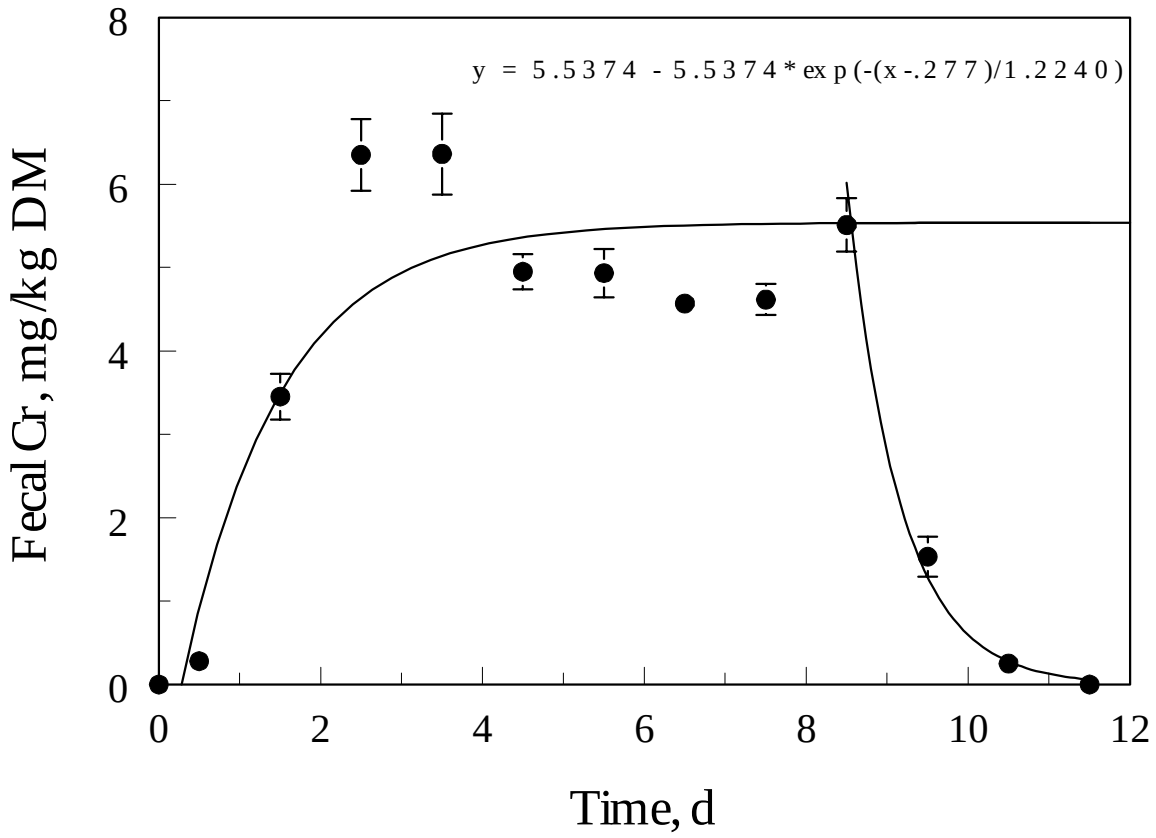

Figure 17.

Mean daily fecal concentrations of Cr, C_t, for horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Cr) are plotted against time, and the data are fit to a one-compartment model.

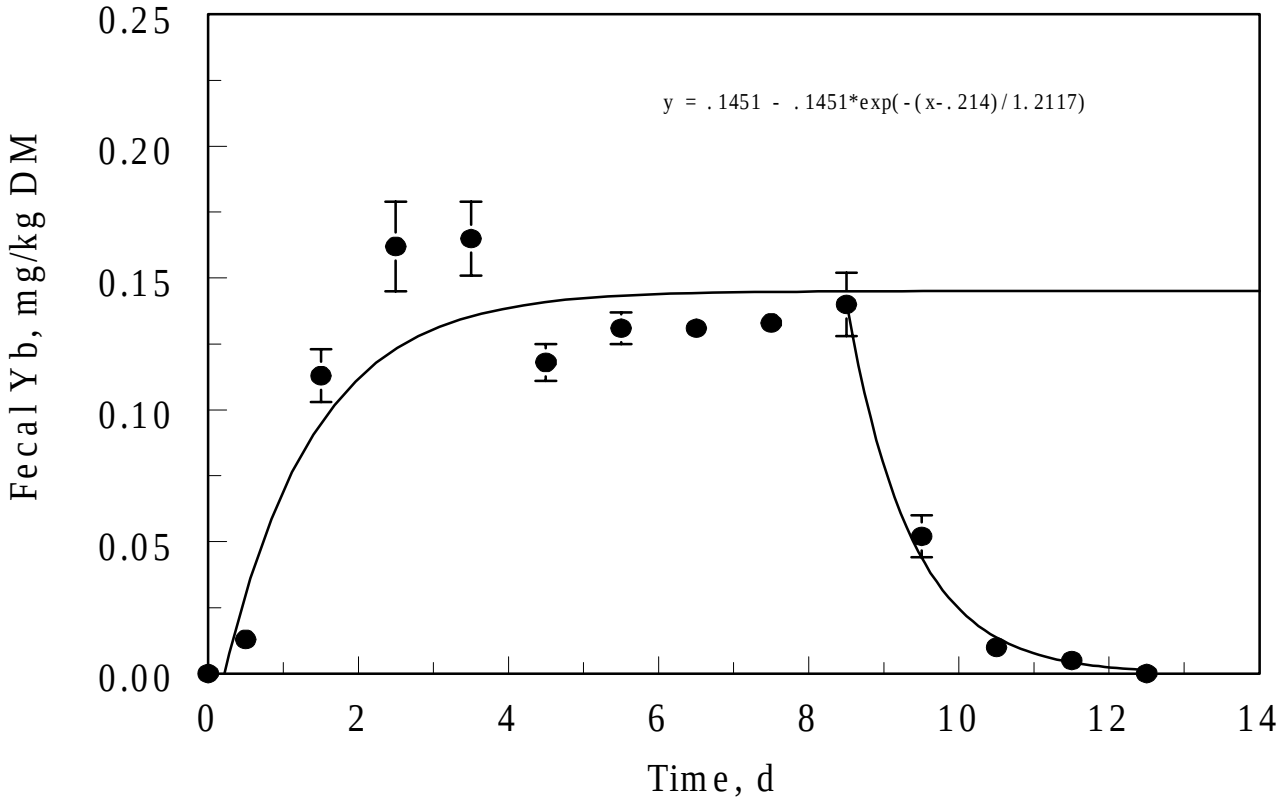

Figure 18.

Mean daily fecal concentrations of Yb, C_t, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Yb) are plotted against time, and the data are fit to a one-compartment model.

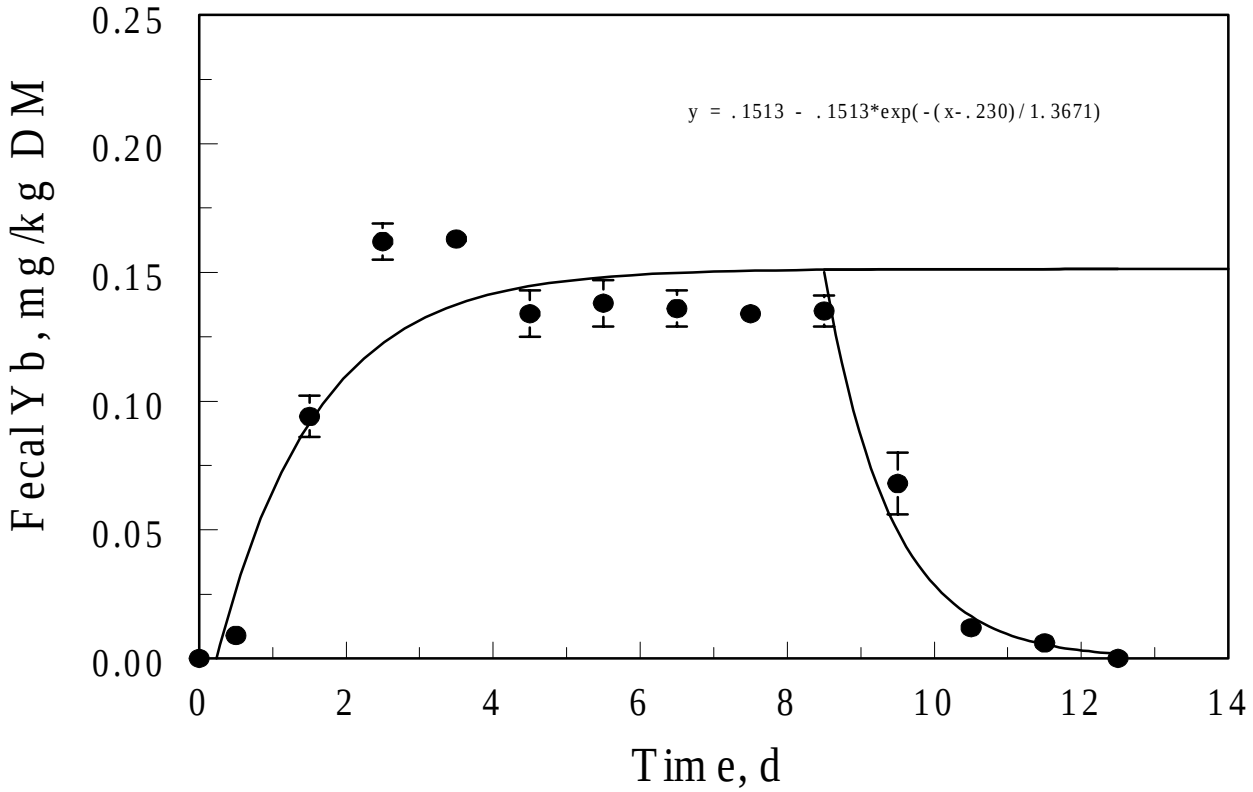

Figure 19.

Mean daily fecal concentrations of Yb, C_t, for horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Yb) are plotted against time, and the data are fit to a one-compartment model.

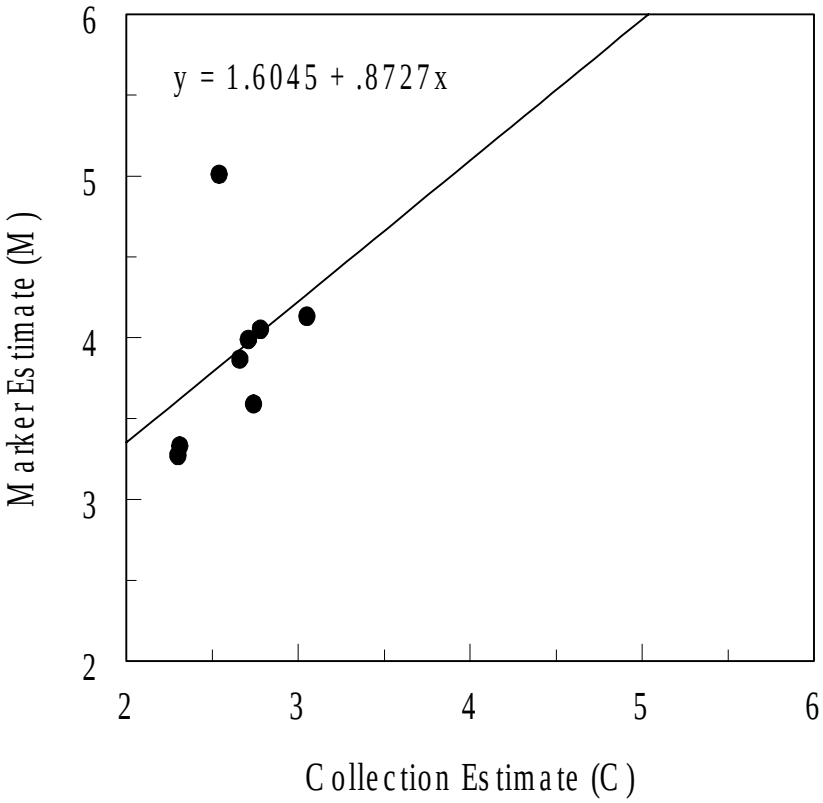

Figure 20. Linear relationship between total collection (C) fecal output and corresponding marker estimates (M), predicted by Cr marker dilution, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Cr) in Experiment 2.

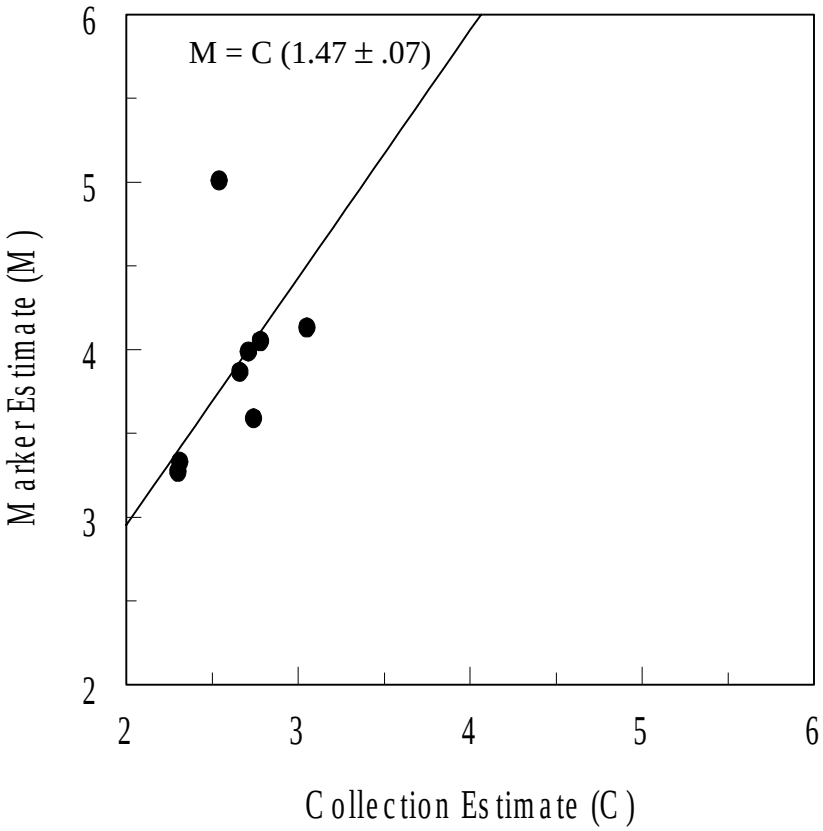

Figure 21. Total collection (C) fecal output and corresponding marker estimates (M), predicted by chromium marker dilution, were correlated for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Cr) in Experiment 2.

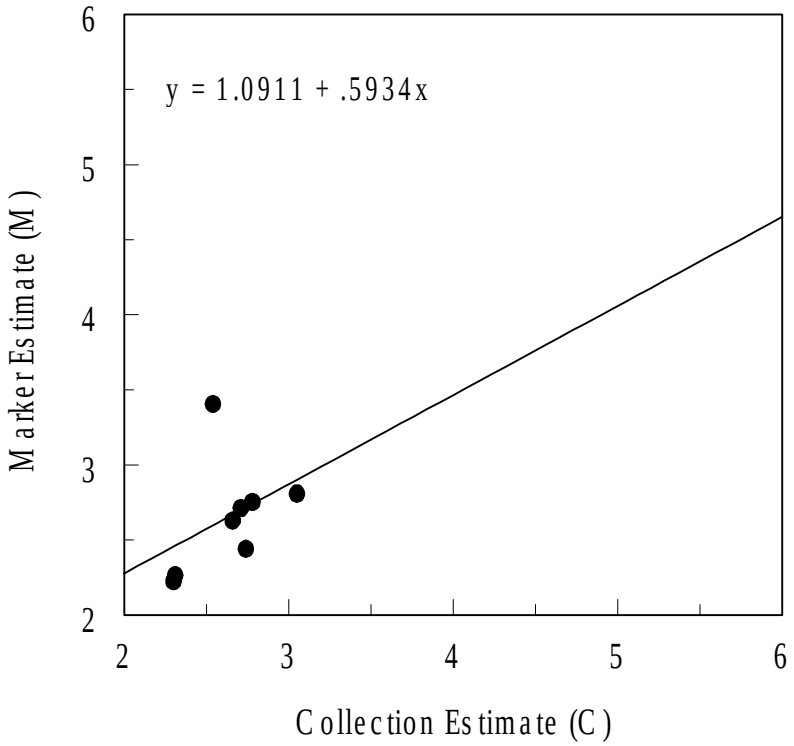

Figure 22. Linear relationship between total collection (C) fecal output and corresponding adjusted marker estimates (M) by chromium marker dilution, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Cr) in Experiment 2.

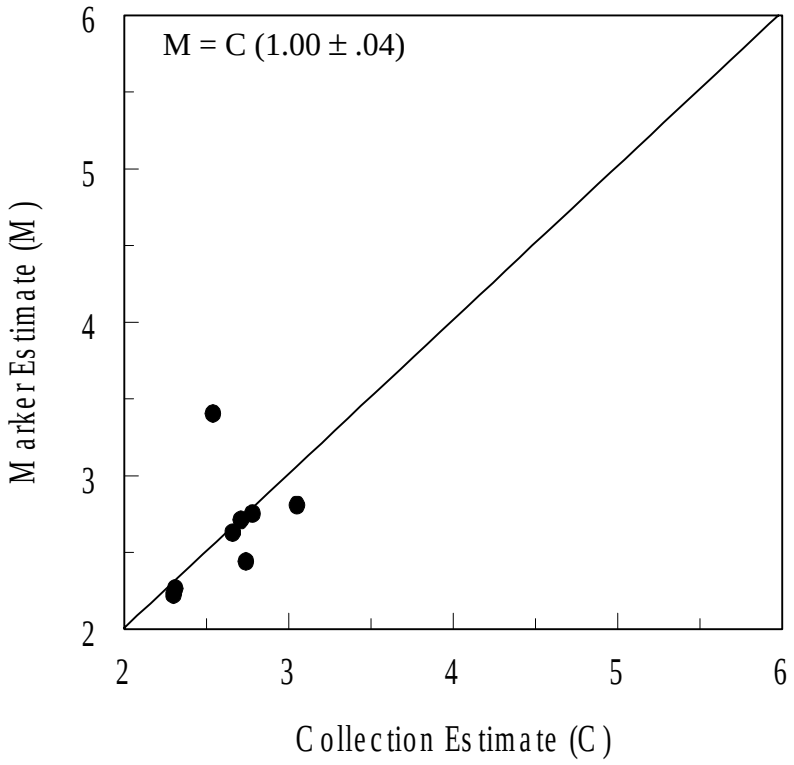

Figure 23. Total collection (C) fecal output and adjusted marker estimates (M) predicted by chromium marker dilution, were correlated for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Cr) in Experiment 2.

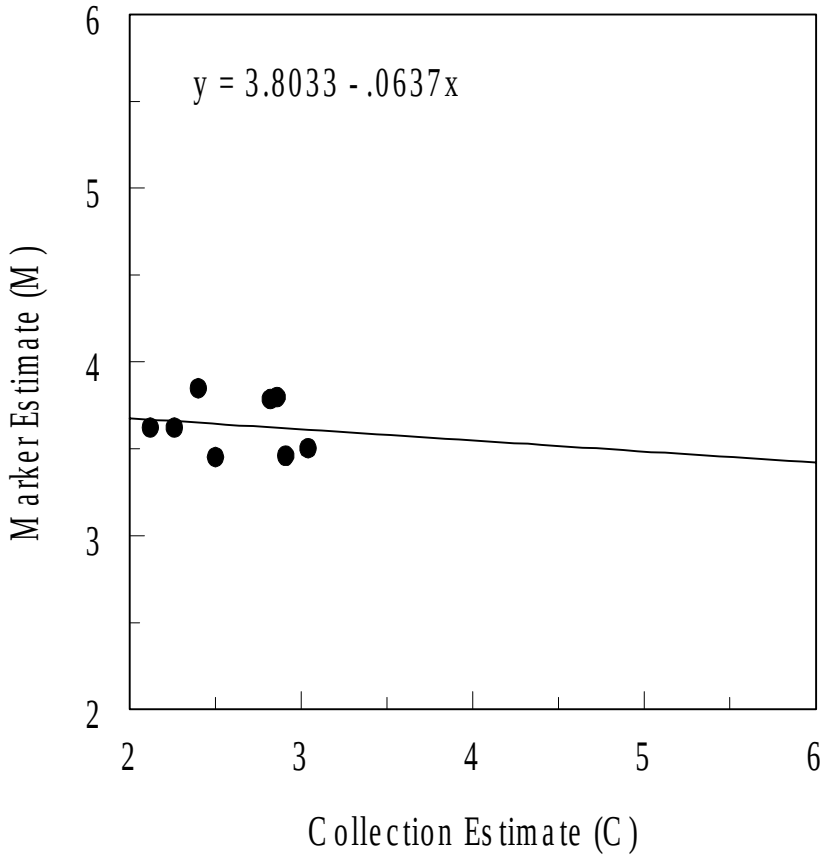

Figure 24. Linear relationship between total collection (C) fecal output and corresponding marker estimates (M) by chromium marker dilution, for horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Cr) in Experiment 2.

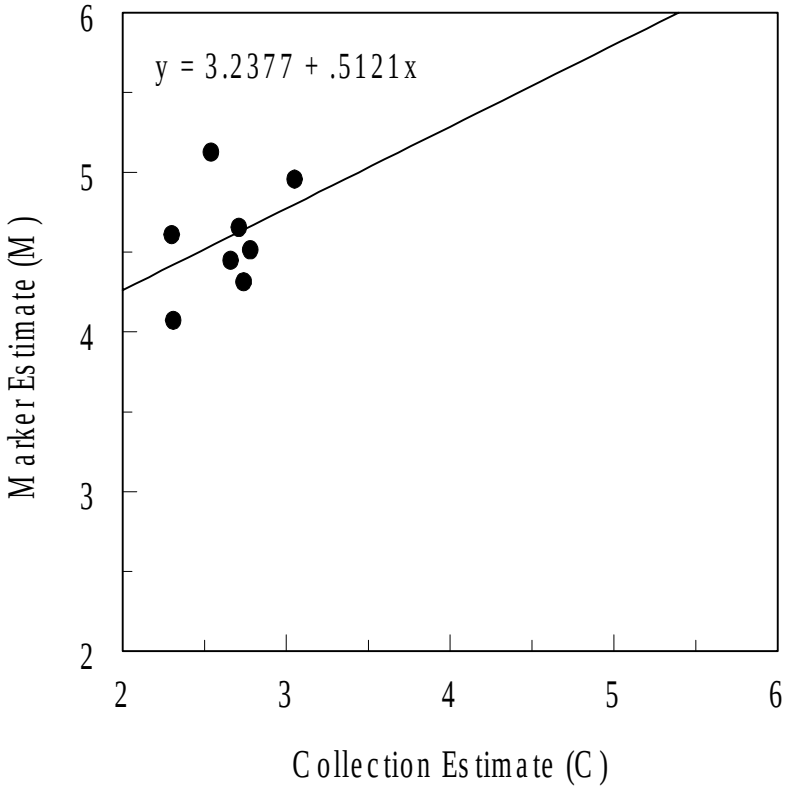

Figure 25. Linear relationship between total collection (C) fecal output and corresponding marker estimates (M) by ytterbium marker dilution, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Yb) in Experiment 2.

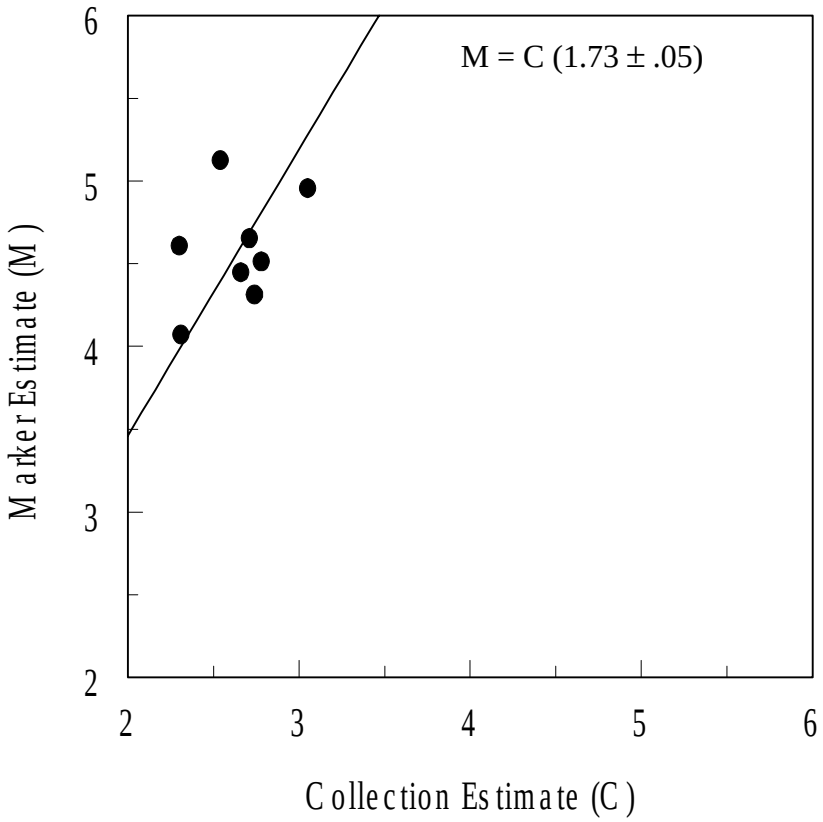

Figure 26. Total collection (C) fecal output and corresponding marker estimates (M) by ytterbium marker dilution, were correlated for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Yb) in Experiment 2.

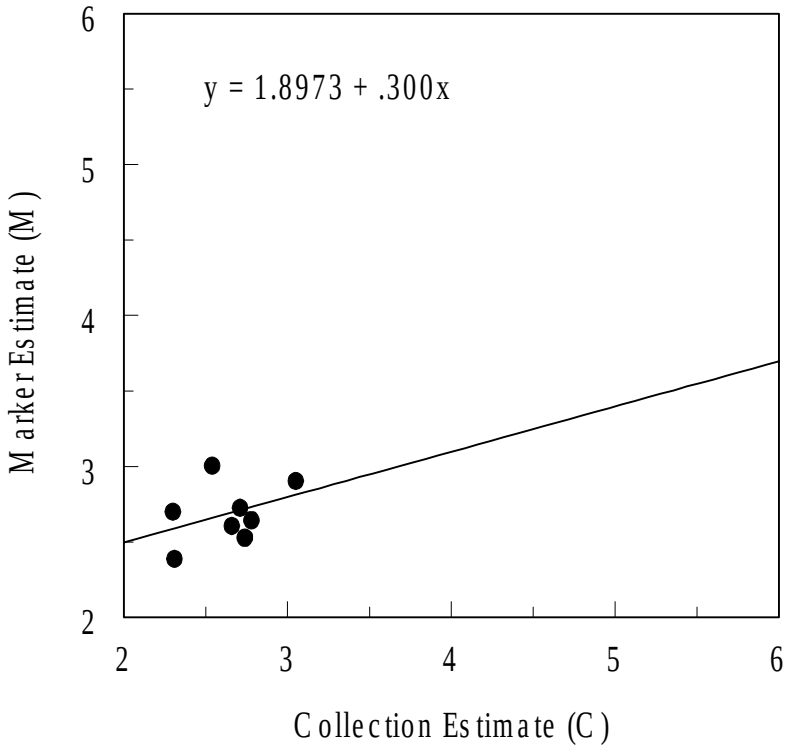

Figure 27. Linear relationship between total collection (C) fecal output and corresponding adjusted marker estimates (M) predicted by ytterbium marker dilution, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Yb) in Experiment 2.

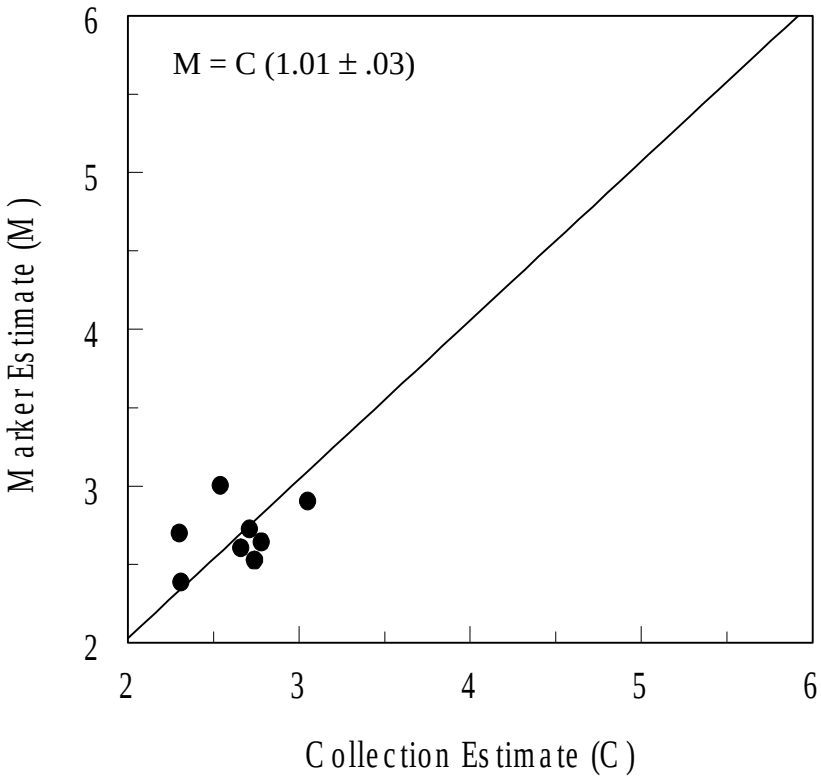

Figure 28. Total collection (C) fecal output and adjusted marker estimates (M) predicted by ytterbium marker dilution, were correlated for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Yb) in Experiment 2.

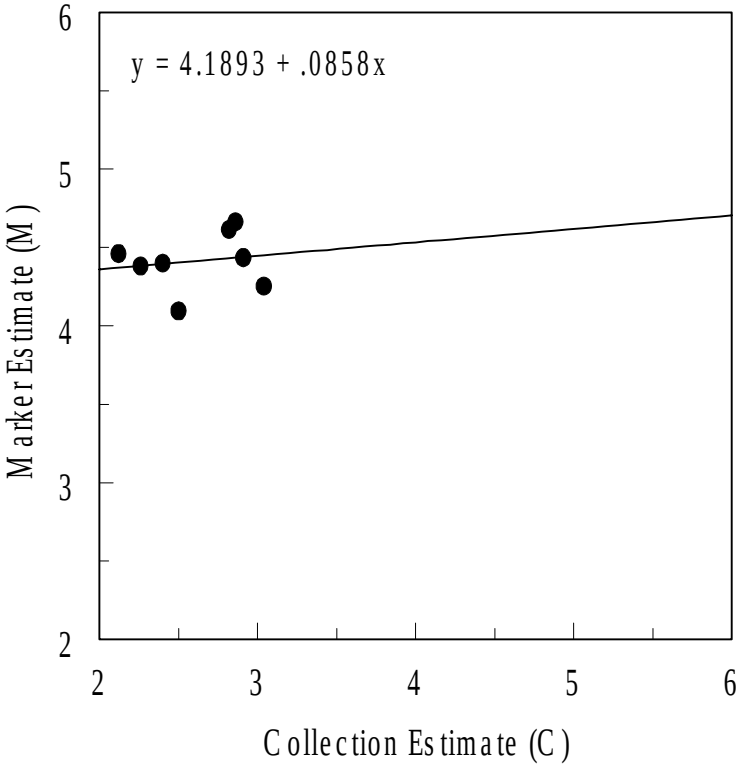

Figure 29. Linear relationship between total collection (C) fecal output and corresponding marker estimates (M) by ytterbium marker dilution, for horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Yb) in Experiment 2.

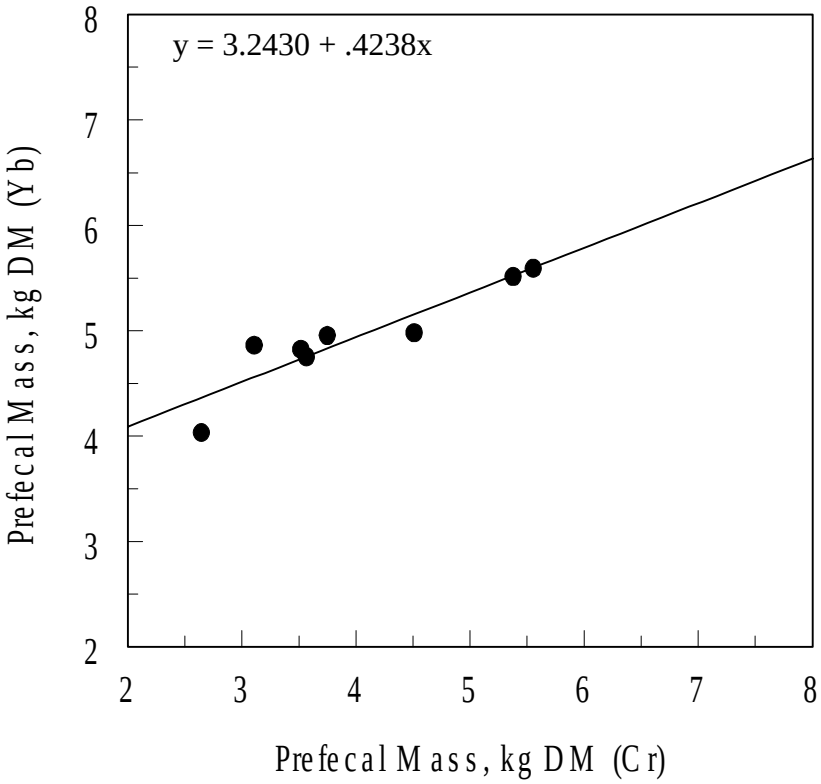

Figure 30. Linear relationship between prefecal mass (kg DM) predicted by chromium marker dilution (Cr) and prefecal mass predicted by ytterbium marker dilution (Yb), for horses offered orchardgrass/alfalfa hay plus fat-and-fiber supplement (Diet 3Cr and 3Yb), in Experiment 2.

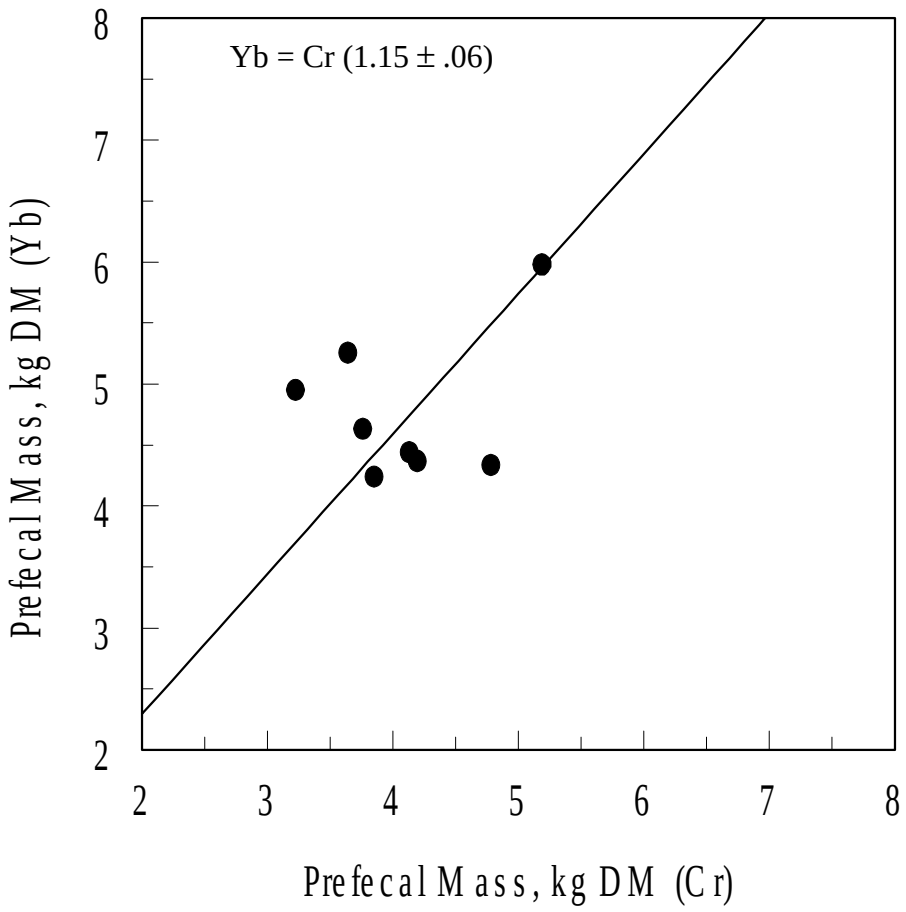

Figure 31. Prefecal masses predicted by chromium marker dilution and prefecal masses predicted by ytterbium marker dilution values, were correlated for horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diets 4Cr and 4Yb), in Experiment 2.

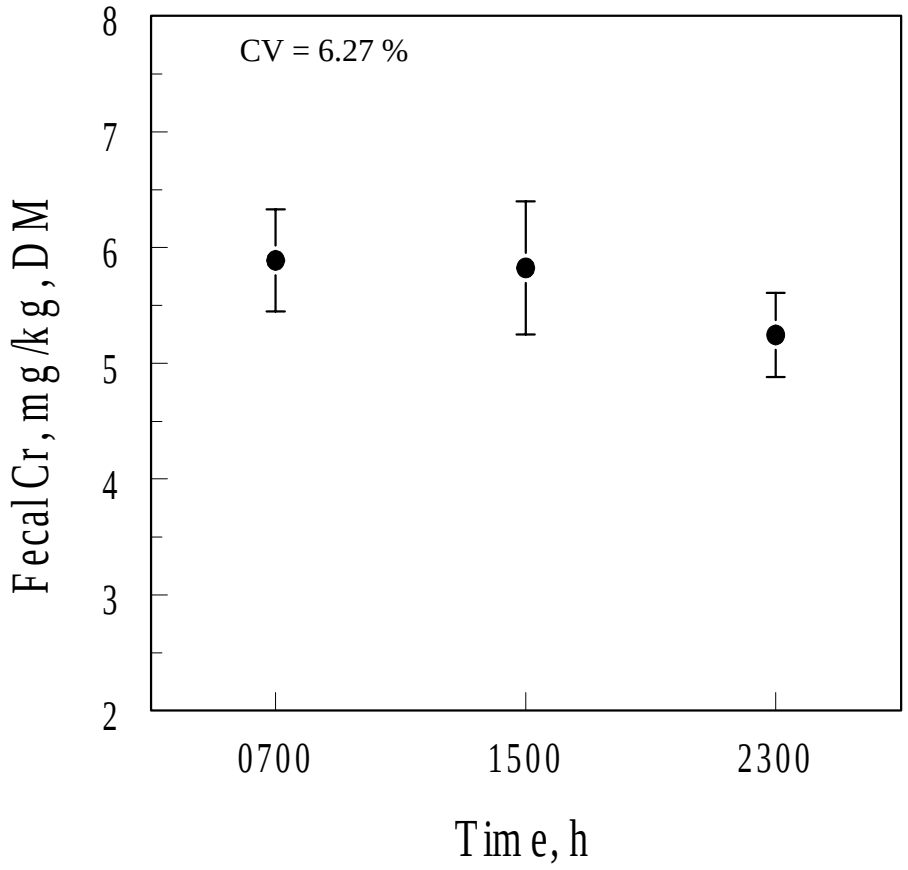

Figure 32. Diurnal variation in fecal concentration (mg/kg, DM) of chromium for individual horse fecal grab samples collected on d 8 of dosing, for horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Cr) in Experiment 2.

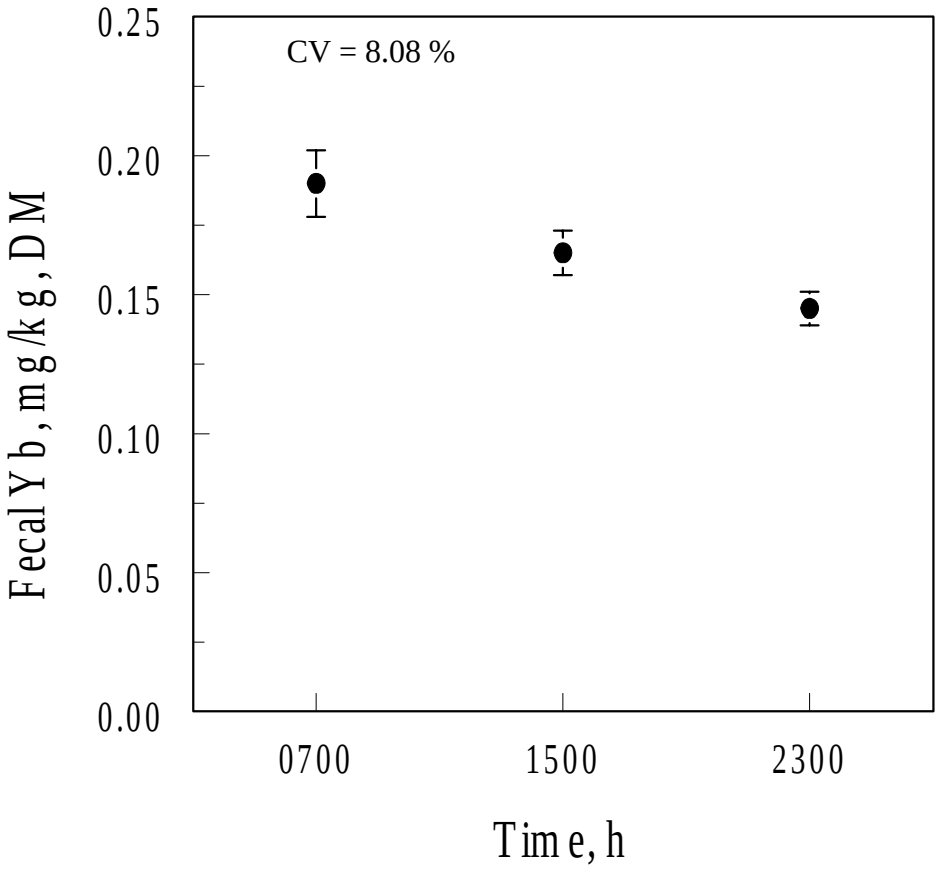

Figure 33. Diurnal variation in fecal concentration (mg/kg, DM) of ytterbium for individual horse fecal grab samples collected on d 8 of dosing, for horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Yb) in Experiment 2.

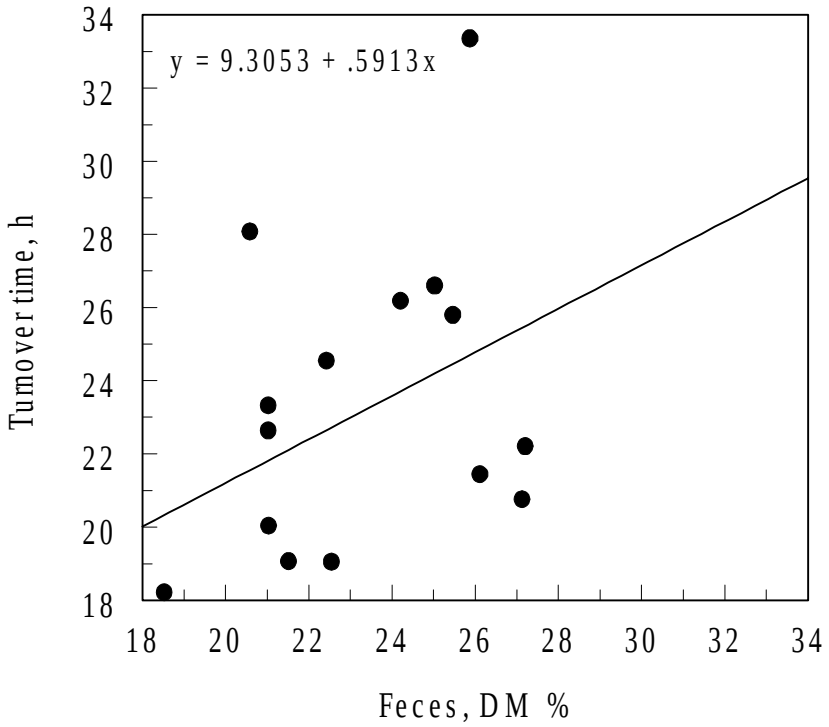

Figure 34. Linear relationship between dry matter percentage of feces (DM, %) and turnover times predicted by chromium marker dilution, for horses offered orchardgrass/alfalfa hay (Diet 1), in Experiment 1, and horses offered orchardgrass/alfalfa hay plus fat-and-fiber supplement (Diet 3Cr), in Experiment 2.

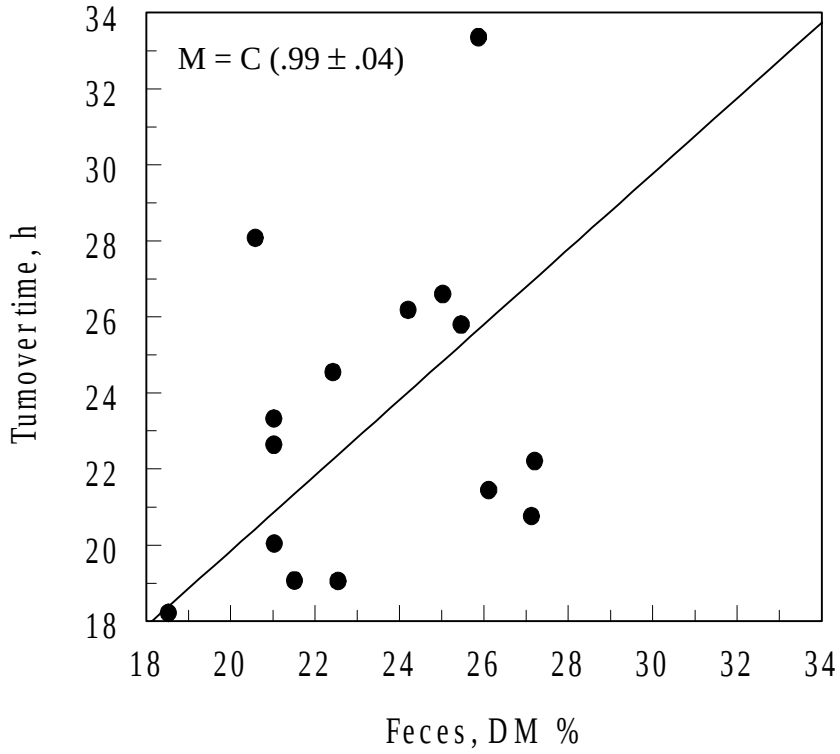

Figure 35. Dry matter percentage of feces (DM %) were correlated with turnover times predicted by chromium marker dilution, for horses offered orchardgrass/alfalfa hay (Diet 1) in Experiment 1, and horses offered orchardgrass/alfalfa hay plus fat-and-fiber supplement (Diet 3Cr) in Experiment 2.

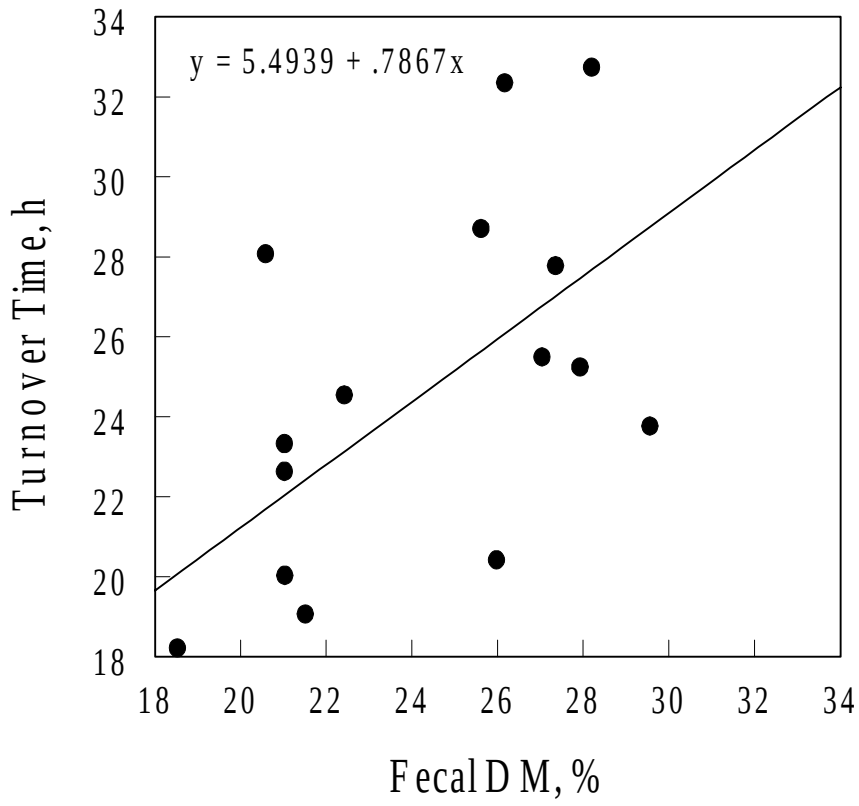

Figure 36. Linear relationship between percentage DM of feces and corresponding turnover times predicted by ytterbium marker dilution, for horses offered orchardgrass/alfalfa hay (Diet 1) and for horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Yb), in Experiment 1 and 2, respectively.

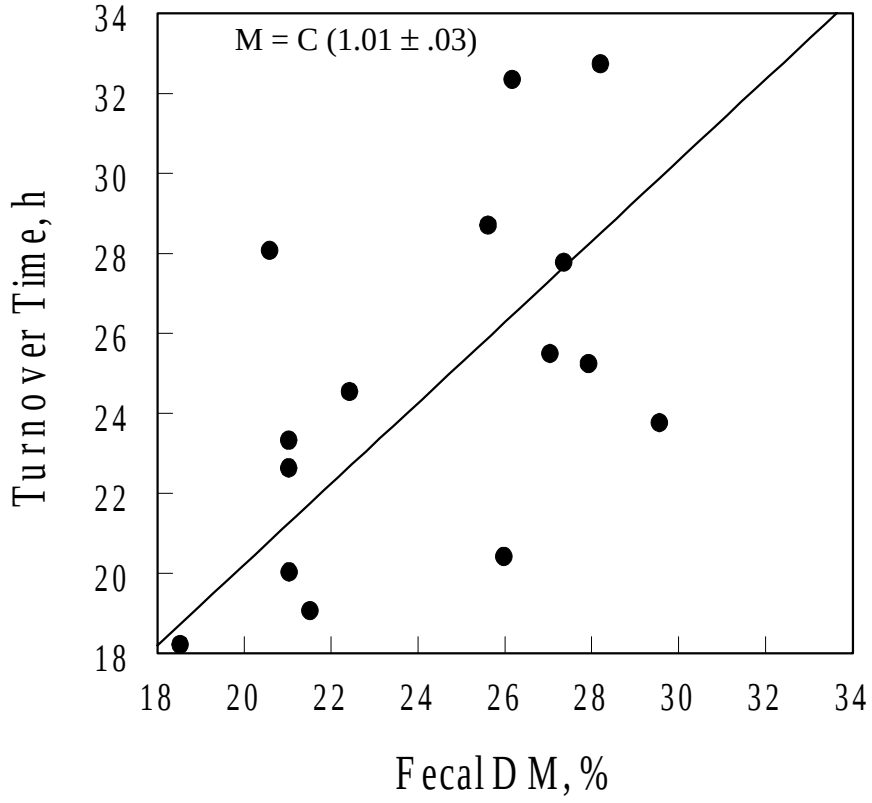

Figure 37. Dry matter percentage of feces (DM %) and corresponding turnover times predicted by chromium marker dilution, were correlated for horses offered orchardgrass/alfalfa hay (Diet 1) and for horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Cr) in Experiment 1 and 2, respectively.

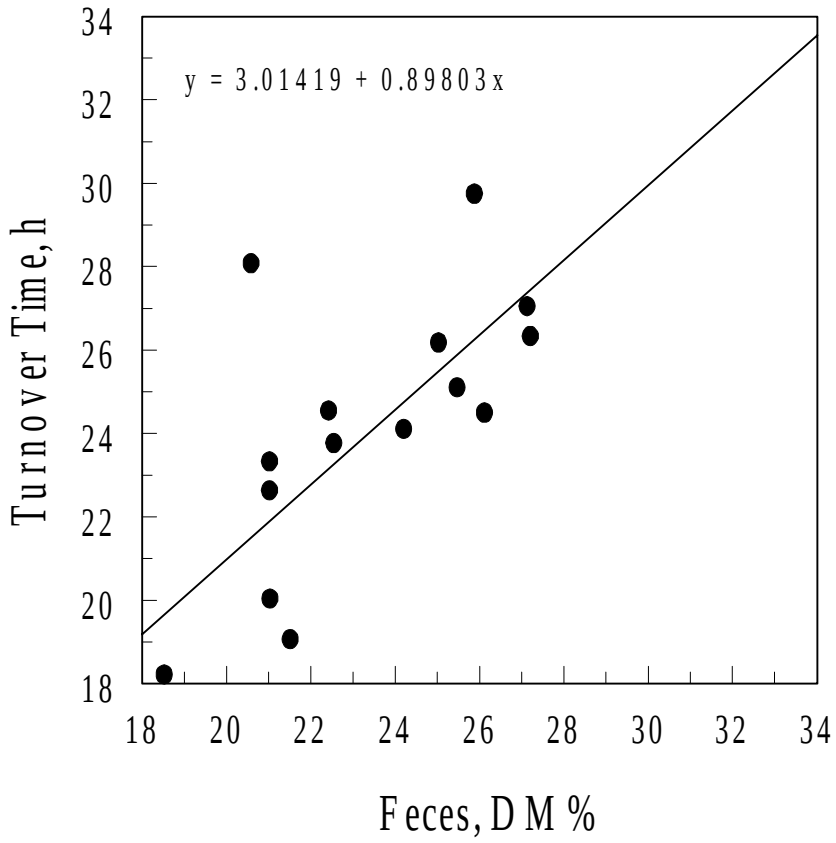

Figure 38. Linear relationship between percentage DM of feces and corresponding turnover times predicted by ytterbium marker dilution, for horses offered orchardgrass/alfalfa hay (Diet 1) and for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Yb) in Experiment 1 and 2, respectively.

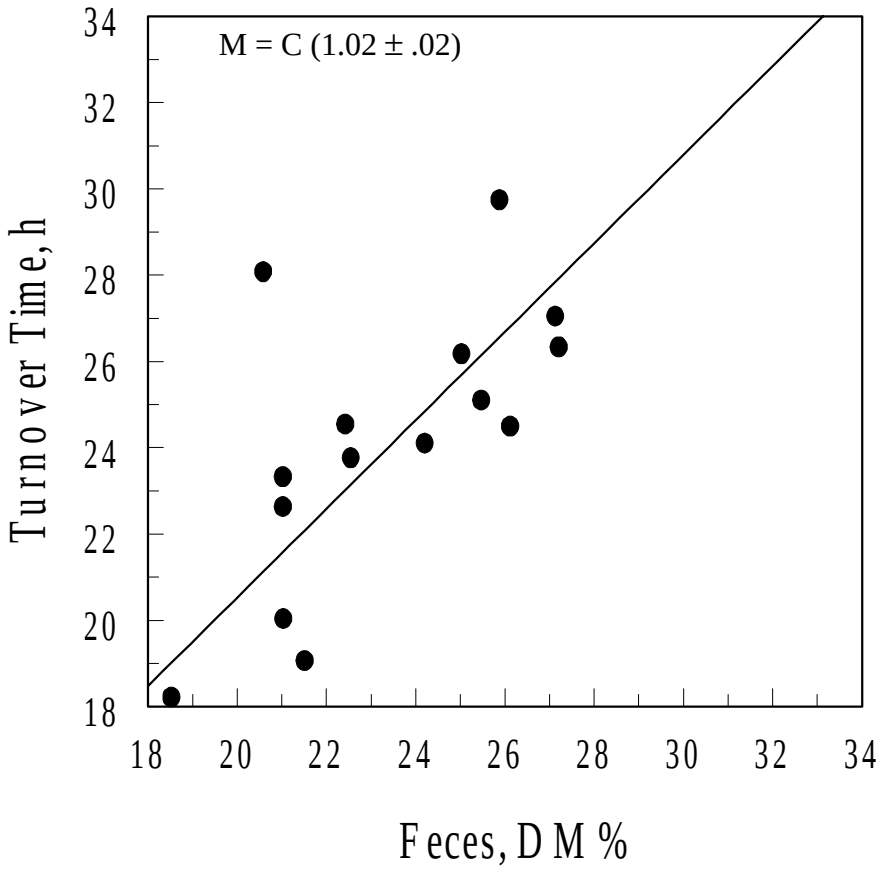

Figure 39. Percentage DM of feces (DM %) and corresponding turnover times predicted by ytterbium marker dilution, were correlated for horses offered orchardgrass/alfalfa hay (Diet 1), and orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Yb) in Experiment 1 and 2, respectively.

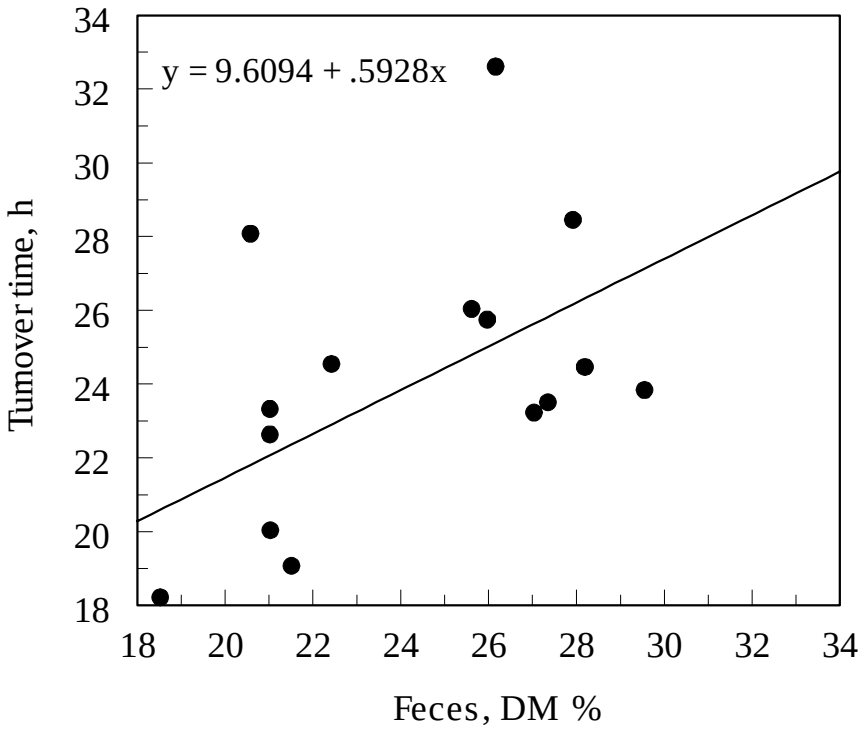

Figure 40. Linear relationship between dry matter percentage of feces (DM, %) and turnover times for horses offered orchardgrass/alfalfa hay (Diet 1), predicted by chromium marker dilution in Experiment 1, and horses offered orchardgrass/alfalfa hay plus sugar-and-starch supplement (Diet 4Yb), predicted by ytterbium marker dilution in Experiment 2.

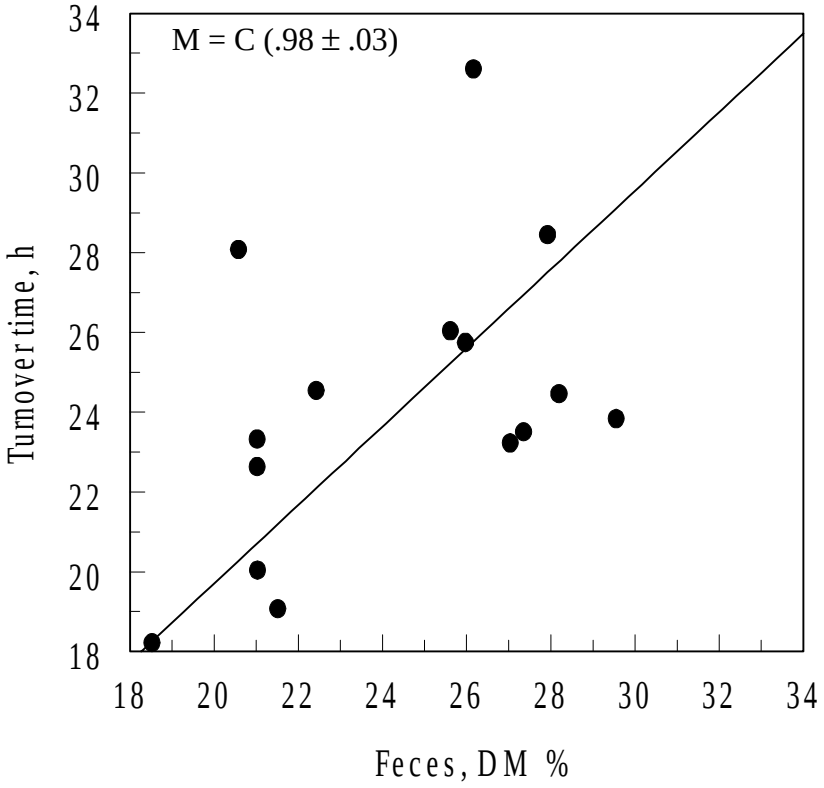

Figure 41. Dry matter percentage of feces (DM %) were correlated with turnover times for horses offered orchardgrass/alfalfa hay (Diet 1), predicted by chromium marker dilution in Experiment 1, and horses offered orchardgrass/alfalfa hay plus sugar-and-starch supplement (Diet 4Yb) predicted by ytterbium marker dilution in Experiment 2.

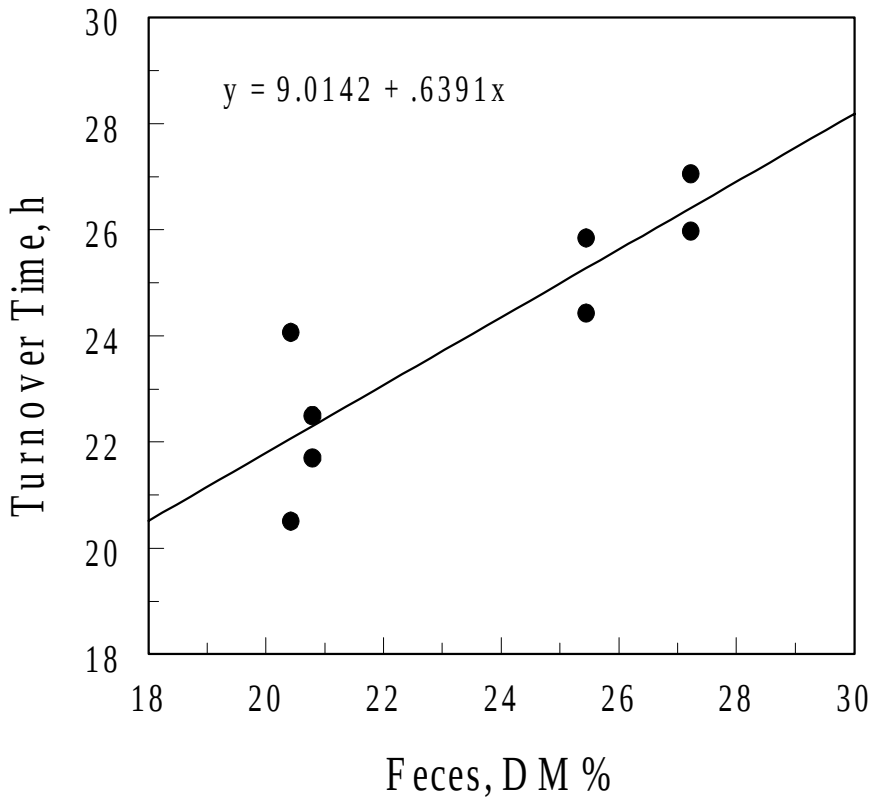

Figure 42. Linear relationship between mean percentage dry matter of feces (DM, %) and corresponding mean turnover times predicted by chromium marker dilution, for horses offered hay only in Experiment 1, and hay and supplement in Experiment 2.

Appendix Table 1. Dietary treatments for horses offered orchardgrass/alfalfa hay (Diet 1) or tall fescue/alfalfa hay (Diet 2) in Experiment 1

	Diet	
Horse	Period 1[a]	Period 2[b]
1	1	2
2	2	1
3	1	2
4	2	1
5	2	1
6	1	2
7	1	2
8	2	1

[a] February 1997

[b] March 1997

Appendix Table 2. Nutrient composition on a DM basis of orchardgrass/alfalfa hay (Diet 1) and tall fescue/alfalfa hay (Diet 2) in Experiment 1[a,b]

	Diet 1		Diet 2	
Item	Mean	SE	Mean	SE
Crude Protein, %	14.3	.08	11.8	.54
Crude Fat, %	.83	.03	1	.22
Acid Detergent Fiber, %	47.5	.85	48.4	.76
Neutral Detergent Fiber, %	62.6	.58	66.2	1.1
Non-Structural Carbohydrates, %	14.2	.46	13.8	13.8
Ash, %	8	.1	7.2	7.2
TDN, %	53	0	53	.41
DE, Mcal/kg	2	.01	1.9	.02
Calcium, %	.72	.02	.61	.02
Phosphorus, %	.3	.01	.25	.003
Magnesium, %	.16	.01	.18	.004
Pottasium, %	3	.03	2.6	.09
Sodium, %	.01	.002	.012	.002
Iron, mg/kg	605	114	645	72
Zinc, mg/kg	36	3.2	36	2.6
Copper, mg/kg	10	.7	10	.6
Sulfur, %	.16	.04	.16	.86

[a] Analysis performed by Dairy One, Ithaca, NY;
[b] (n = 4)

Appendix Table 3. Dietary treatments for horses offered orchardgrass/alfalfa hay and FF (Diet 3) or orchardgrass/alfalfa hay and SS (Diet 4) in Experiment 2

	Diet	
Horse	Period 1[a]	Period 2[b]
1	1	2
2	2	1
3	1	2
4	2	1
5	2	1
6	1	2
7	1	2
8	2	1

[a] Mid-April to mid-May 1997

[b] July 1997

Appendix Table 4. Nutrient composition on a DM basis of orchardgrass/alfalfa hay (OG), FF supplement (Diet 3) and SS supplement (Diet 4) in Experiment 2[a,b]

	OG		FF		SS	
Item	Mean	SE	Mean	SE	Mean	SE
Crude Protein, %	13.4	.1	18.4	1	14	.1
Crude Fat, %	1.9	.2	9.6	1.1	6.9	.2
Acid Detergent Fiber, %	46.5	.9	26.3	1.4	8.7	.2
Neutral Detergent Fiber, %	64.5	1.9	38.1	.5	18	.4
Non-Structural Carbohydrates, %	12.5	1.8	25.8	.2	56	.3
Ash, %	7.8	.5	8.2	.5	5.1	.04
TDN, %	56	.48	77	1.2	82	.41
DE, Mcal/kg	2	.01	2.63	.08	3.60	.01
Calcium, %	.67	.03	1.40	.10	.58	.01
Phosphorus, %	.27	.00	.68	.03	.62	.00
Magnesium, %	.16	.01	.23	.00	.18	.01
Pottasium, %	2.5	.24	1.40	.10	.86	.01
Sodium, %	.02	.01	.30	.03	.21	.01
Iron, mg/kg	550	139	698	11	605	34
Zinc, mg/kg	48	9.2	164	9.7	185	3.4
Copper, mg/kg	11.5	1	34	2.1	38	2
Sulfur, %	.15	.01	.20	.01	.19	.02

[a] Analysis performed by Dairy One, Ithaca, NY;
[b] (n = 4)

Appendix Table 5. Ingredient composition (%) of the fat-and-fiber (FF) supplement offered in Experiment 2

Ingredient	FF
Corn dent yellow grain	4
Soybean meal	22
Oat straw	23
Soybean hulls	15
Beet pulp	16.5
Cane Molasses	5
Corn oil	11
Calcium phos dibasic	1.7
Limestone	.8
Mineral premix[a]	.5
Vitamin premix[b,c]	.5

[a] Provided the following amounts per kg of diet: Fe, 46.1 mg;

[b] Courtesy of Hoffman-LaRoche Nutely, NJ

[c] Provided the following amounts per kg of diet: Vitamin A, 6900 IU; β-carotene, 17.6 IU; Vitamin D_3, 1290 IU; Vitamin E, 132 mg; Vitamin C, 333 mg; Niacin, 15 mg; Thiamin, 7 mg; Riboflavin, 3.5 mg; Folic acid, .33 mg; Biotin, .21 mg.

Appendix Table 6. Ingredient composition of the chromium granola bars (Cr_{GB}) offered to horses in Experiment 1 and 2[a]

Ingredient	Cr_{GB}
Oat-based sweet feed	2.2 kg
Chromium sesquioxide[b]	800 g
Cane Molasses	1 L
Beer	380 ml

[a] Adapted from Practical Horseman (April, 1996) by J. L. Holland and D. S. Kronfeld

[b] Fisher Scientific, Fair Lawn, NJ

Appendix Table 7. Dry matter intake (DMI, kg/d), total fecal output (FO, kg/d), and total collection DMD (D_{TC}, %) for horses offered orchardgrass/alfalfa hay (Diet 1) and tall fescue/alfalfa hay (Diet 2) in Experiment 1

	Diet 1			Diet 2		
Horse	DMI	FO	D_{TC}	DMI	FO	D_{TC}
1	10.0	3.83	61.91	9.6	3.86	60.37
2	9.8	3.25	66.67	9.5	4.39	53.97
3	8.5	2.97	65.03	6.8	2.74	59.95
4	12.0	4.74	60.78	9.6	4.23	56.06
5	11.4	4.56	59.84	11.1	4.73	57.39
6	10.7	4.12	61.37	11.0	3.96	64.06
7	7.7	2.71	64.90	7.5	3.01	60.12
8	11.4	4.84	57.49	10.3	4.68	54.65
Mean ± SE[a]	10.2 ± .5[b]	3.9 ± .3[c]	62.2 ± 1.1[d]	9.4 ± .5[b]	3.9 ± .3[c]	58.3 ± 1.2[d]

[a] Standard error of the mean (n = 8)

[b] (P = .390); [c] (P = .791); [d] (P = .079)

Appendix Table 8. Fecal concentrations (mg/kg) of yttrium for horses offered orchardgrass/alfalfa hay (Diet 1) and tall fescue/alfalfa hay (Diet 2) in Experiment 1

Horse	Diet 1	Diet 2
1	2.95	1.98
2	3.04	1.48
3	3.26	1.90
4	3.19	2.01
5	3.18	2.09
6	2.07	1.42
7	3.73	1.95
8	2.66	1.62
Mean ± SE[a]	3.01 ± .17[b]	1.81 ± .09[b]

[a] Standard error of the mean (n = 8)

[b] (P = .001)

Appendix Table 9. Mean daily fecal concentrations (mg/kg, DM) of chromium for horses offered orchardgrass/alfalfa hay (Diet 1a) and tall fescue/alfalfa hay (Diet 2a) using total collection data in Experiment 1[a]

Diet 1a			Diet 2a		
Mean			Mean		
Day	Cr mg/kg DM	SE[b]	Day	Cr mg/kg DM	SE[b]
1	0	0	1	0	0
2	0	0	2	0	0
3	0	0	3	0	0
4	204.5	104.9	4	405.8	157.7
5	4151.9	333.0	5	4076.4	369.9
6	5720.3	487.4	6	5013.8	388.5
7	5354.4	496.8	7	5305.8	368.1
8	3646.6	213.6	8	3490.2	417.7
9	3441.8	310.3	9	4302.5	369.3
10	4568.6	398.4	10	4690.2	397.2
11	4761.1	570.6	11	4834.4	305.3
12	4536.9	591.0	12	5247.9	587.7
13	844.4	275.7	13	788.6	192.5
14	0	0	14	0	0
15	0	0	15	0	0

[a] Daily fecal Cr concentrations used to generate fecal kinetic models
[b] Standard error of the mean (n = 8)

Appendix Table 10. Mean daily fecal concentrations (mg/kg, DM) of chromium for horses offered orchardgrass/alfalfa hay (Diet 1b) and tall fescue/alfalfa hay (Diet 2b) using fecal grab data in Experiment 1[a]

Diet 1b			Diet 2b		
Mean			Mean		
Day	Cr mg/kg DM	SE[b]	Day	Cr mg/kg DM	SE[b]
1	0	0	1	0	0
2	0	0	2	0	0
3	0	0	3	0	0
4	204.5	104.9	4	405.8	157.7
5	4151.9	333.0	5	4076.4	369.9
6	5720.3	487.4	6	5013.8	388.5
7	5354.4	496.8	7	5305.8	368.1
8	3646.6	213.6	8	3490.2	417.7
9	4098.3	419.5	9	3725.3	284.9
10	3632.3	293.8	10	3659.7	296.4
11	3533.9	288.8	11	3403.8	255.2
12	4138.1	653.9	12	3218.9	264.7
13	844.4	275.7	13	788.6	192.5
14	0	0	14	0	0
15	0	0	15	0	0

[a] Daily fecal Cr concentrations used to generate fecal kinetic models
[b] Standard error of the mean (n = 8)

Appendix Table 11. Individual horse daily fecal concentrations (mg/kg, DM) of chromium for horses offered orchardgrass/alfalfa hay (Diet 1a) using total collection data in Experiment 1[a]

	Horse							
Day	1	2	3	4	5	6	7	8
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	542.5	717.5	0	376.3
5	3920	3710	3666.3	2835	4025	5355	5731.3	3972.5
6	5670	6860	5941.3	3622.5	6002.5	4007.5	7848.8	5810
7	5792.5	6168	7192.5	5022.5	5040	4401.3	6527.5	2690.6
8	3832.5	4445	4130	3167.5	3080	3237.5	4322.5	2957.5
9	5915	222.5	3071.2	3412.5	3158.7	5720	3797.5	2250.2
10	3937.5	5425	4427	4576.2	3578.7	6055	4751.2	3193.7
11	4917.5	3920	3622.5	4856.3	2747.5	5775	4103.7	3500
12	3798	3727.5	3718.7	2511.2	2887.5	5162.5	3552.5	6230
13	1837.5	726.25	2165.6	463.75	323.75	0	993.13	245
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0

[a] Daily fecal Cr concentrations fitted to main OGTC fecal kinetic model

Appendix Table 12. Individual horse daily fecal concentrations (mg/kg, DM) of chromium for horses offered tall fescue/alfalfa hay (Diet 2a) using total collection data in Experiment 1[a]

	Horse							
Day	1	2	3	4	5	6	7	8
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	853.1	805.0	603.8	0	984.4
5	5162.5	4182.5	2380.0	3360.0	5110.0	4987.5	4392.5	3036.3
6	4812.5	4786.3	6142.5	4130.0	3605.0	6037.5	6545.0	4051.3
7	6335.0	5503.8	6107.5	4488.8	3955.0	6693.8	5250.0	4112.5
8	3447.5	2257.5	6055.0	2808.8	2686.3	3500.0	4103.8	3062.5
9	3902.5	4302.1	4515.0	3928.7	3640.0	4217.5	4620.0	3561.3
10	3451.5	5197.5	6142.5	3272.5	4996.2	5447.5	3578.7	6308.7
11	4742.5	6065.0	4332.5	5215.0	5425.0	6231.0	4926.0	4340.0
12	4733.7	6544.8	6255.5	3106.2	5057.5	6234.0	6155.0	5230.0
13	945.0	463.8	1618.8	284.4	1277.5	157.5	1207.5	354.4
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0

[a] Daily fecal Cr concentrations fitted to main TFTC fecal kinetic model

Appendix Table 13. Individual horse daily fecal concentrations (mg/kg, DM) of chromium for horses offered orchardgrass/alfalfa hay (Diet 1b) using fecal grab data in Experiment 1[a]

	Horse							
Day	1	2	3	4	5	6	7	8
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	542.5	717.5	0	376.3
5	3920	3710	3666.3	2835	4025	5355	5731.3	3972.5
6	5670	6860	5941.3	3622.5	6002.5	4007.5	7848.8	5810
7	5792.5	6168	7192.5	5022.5	5040	4401.3	6527.5	2690.6
8	3832.5	4445	4130	3167.5	3080	3237.5	4322.5	2957.5
9	4322.5	5390	5320	3412.5	3202.5	2905	5521.3	2712.5
10	3701.3	4173.8	5302.5	3080	2861.3	3631.3	3596.3	2712.5
11	3876.3	3990	4786.3	2633.8	2467.5	3823.8	3928.8	2765
12	5617.5	3880	6230	2800	2353.8	2870	6930	2423.8
13	1837.5	726.25	2165.6	463.75	323.75	0	993.13	245
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0

[a] Daily fecal Cr concentrations fitted to main OGGR fecal kinetic model

Appendix Table 14. Individual horse daily fecal concentrations (mg/kg, DM) of chromium for horses offered tall fescue/alfalfa hay (Diet 2b) using fecal grab data in Experiment 1[a]

	Horse							
Day	1	2	3	4	5	6	7	8
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	853.1	805.0	603.8	0	984.4
5	5162.5	4182.5	2380.0	3360.0	5110.0	4987.5	4392.5	3036.3
6	4812.5	4786.3	6142.5	4130.0	3605.0	6037.5	6545.0	4051.3
7	6335.0	5503.8	6107.5	4488.8	3955.0	6693.8	5250.0	4112.5
8	3447.5	2257.5	6055.0	2808.8	2686.3	3500.0	4103.8	3062.5
9	3333.8	3053.8	5530.0	3036.3	3482.5	3727.5	4077.5	3561.3
10	3832.5	2896.3	4742.5	2852.5	3517.5	4550.0	4331.3	2555.0
11	3675.0	2257.5	4322.5	2730.0	2992.5	3745.0	4217.5	3290.0
12	3202.5	4208.8	3998.8	2887.5	2852.5	2170.0	3920.0	2511.3
13	945.0	463.8	1618.8	284.4	1277.5	157.5	1207.5	354.4
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0

[a] Daily fecal Cr concentrations fitted to main TFGR fecal kinetic model

Appendix Table 15.

Fits of data to a one-compartment model of fecal kinetics without a delay, the administration curve with a delay, the post-administration curve, and the total curve with a delay between administration of an oral dose of Cr and its entry into the compartment, in horses offered orchardgrass/alfalfa hay using the fecal grab data in Experiment 1[a, b, c]

	No Delay			Administration Curve			Post-Administration Curve			Total Curve		
Horse	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value
1	.693	15.83	.0053	.792	26.66	.0013	.539	2.34	.2656	.783	39.74	.0001
2	.675	14.53	.0066	.777	24.34	.0017	.784	7.26	.1145	.829	53.47	.0000
3	.742	20.12	.0029	.821	32.06	.0008	.460	1.71	.3215	.786	40.44	.0001
4	.688	15.46	.0057	.784	25.48	.0015	.919	22.78	.0412	.794	42.48	.0000
5	.539	8.18	.0244	.667	14.02	.0072	.954	41.65	.0232	.737	30.87	.0002
6	.714	17.51	.0041	.764	22.69	.0021	.872	13.64	.0661	.799	43.75	.0000
7	.548	8.50	.0225	.687	15.39	.0057	.392	1.29	.3735	.658	21.15	.0008
8	.507	7.20	.0314	.648	12.88	.0089	.943	32.82	.0291	.711	27.07	.0003

[a] Individual horse data
[b] Delay from main OGGR model (6.192 h)
[c] Model fitted using the chromium external marker

Appendix Table 16.

Fits of data to a one-compartment model of fecal kinetics without a delay, the administration curve with a delay, the post-administration curve, and the total curve with a delay between administration of an oral dose of Cr and its entry into the compartment, in horses offered tall fescue/alfalfa hay using the total collection data in Experiment 1[a, b, c]

	No Delay			Administration Curve			Post-Administration Curve			Total Curve		
Horse	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value
1	.651	13.07	.0086	.724	18.38	.0036	.936	29.41	.0324	.812	47.45	.0000
2	.860	42.95	.0003	.885	54.12	.0002	.792	7.63	.1099	.905	104.19	.0000
3	.792	26.59	.0013	.830	34.29	.0006	.859	12.17	.0733	.884	83.63	.0000
4	.833	34.93	.0006	.856	41.75	.0004	.932	27.47	.0345	.858	66.53	.0000
5	.817	31.21	.0008	.847	38.84	.0004	.964	53.63	.0181	.888	87.65	.0000
6	.859	42.65	.0003	.886	54.17	.0002	.970	65.62	.0149	.908	108.84	.0000
7	.640	12.45	.0096	.707	16.87	.0045	.865	12.84	.0698	.797	43.12	.0000
8	.894	58.88	.0001	.893	58.20	.0001	.965	55.25	.0176	.877	78.63	.0000

[a] Individual horse data
[b] Delay from mean TFTC model (3.144 h)
[c] Model fitted using the chromium external marker

Appendix Table 17.

Fits of data to a one-compartment model of fecal kinetics without a delay, the administration curve with a delay, the post-administration curve, and the total curve with a delay between administration of an oral dose of Cr and its entry into the compartment, in horses offered tall fescue/alfalfa hay using the fecal grab data in Experiment 1[a, b, c]

	No Delay			Administration Curve			Post-Administration Curve			Total Curve		
Horse	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value
1	.560	8.90	.0204	.681	14.97	.0061	.870	13.40	.0672	.764	35.71	.0001
2	.461	5.98	.0445	.596	10.32	.0148	.637	5.26	.1488	.681	23.44	.0005
3	.757	21.83	.0023	.821	32.09	.0008	.746	5.88	.1365	.858	66.53	.0000
4	.769	23.31	.0019	.799	27.91	.0011	.875	14.02	.0645	.827	52.47	.0000
5	.436	5.41	.0529	.769	23.24	.0019	.918	22.49	.0417	.815	48.57	.0000
6	.414	4.94	.0616	.742	20.13	.0028	.694	6.80	.1210	.805	45.48	.0000
7	.685	15.25	.0059	.779	24.72	.0016	.601	4.51	.1676	.821	50.47	.0000
8	.852	40.36	.0004	.839	36.53	.0005	.929	25.98	.0364	.851	63.01	.0000

[a] Individual horse data
[b] Delay from mean TFGR model (4.872 h)
[c] Model fitted with chromium external marker

Appendix Table 18. Estimates of fit of individual fecal chromium concentration data to a one-compartment model, model parameters, and calculated variables in horses offered orchardgrass/alfalfa hay, using fecal grab data (Diet 1b) in Experiment 1

Horse	Cr_a, mg/kg[a]	FO_{CA}, kg/d[b]	TT, h[c]	PFM, kg[d]	R^2	*F*	*P*-value
1	4.585	4.209	23.33	4.091	.792	26.66	.00010
2	5.176	3.728	24.55	3.814	.777	24.34	.00130
3	5.552	3.475	28.08	4.067	.821	32.06	.00076
4	3.768	5.121	22.64	4.832	.794	42.48	.00033
5	4.011	4.811	19.07	3.822	.737	30.87	.00085
6	3.903	4.944	17.68	3.641	.764	22.69	.00265
7	5.372	3.592	20.04	3.000	.687	15.39	.00125
8	3.623	5.326	18.22	4.043	.711	27.07	.00155

[a] Cr_a is the asymptotic value of fecal Cr concentration
[b] FO_{CA} is the fecal output, FO_{CA} = dose of Cr/Cr_a
[c] TT is turnover time of the prefecal mass, TT = -1/k
[d] PFM is prefecal mass or mixing compartment, PFM = $FO_{CA} \bullet$ TT

Appendix Table 19. Estimates of fit of individual fecal chromium concentration data to a one-compartment model, model parameters, and calculated variables in horses offered tall fescue/alfalfa hay, using total collection data (Diet 2a) in Experiment 1

Horse	Cr_a, mg/kg[a]	FO_{CA}, kg/d[b]	TT, h[c]	PFM, kg[d]	R^2	*F*	*P*-value
1	4.840	3.986	23.33	3.383	.812	47.45	.00023
2	5.622	3.432	24.55	5.026	.885	54.12	.00016
3	5.762	3.349	28.08	4.538	.830	34.29	.00063
4	4.278	4.510	22.64	3.078	.858	66.53	.00008
5	4.730	4.080	19.07	3.211	.888	87.65	.00003
6	5.963	3.236	17.68	2.958	.908	108.84	.00002
7	5.016	3.847	20.04	3.688	.707	16.87	.00453
8	5.201	3.710	18.22	3.762	.877	78.63	.00005

[a] Cr_a is the asymptotic value of fecal Cr concentration
[b] FO_{CA} is the fecal output, FO_{CA} = dose of Cr/Cr_a
[c] TT is turnover time of the prefecal mass, TT = -1/k
[d] PFM is prefecal mass or mixing compartment, PFM = $FO_{CA} \bullet$ TT

Appendix Table 20. Estimates of fit of individual fecal chromium concentration data to a one-compartment model, model parameters, and calculated variables in horses offered tall fescue/alfalfa hay, using fecal grab data (Diet 2b) in Experiment 1

Horse	Cr_a, mg/kg[a]	FO_{CA}, kg/d[b]	TT, h[c]	PFM, kg[d]	R^2	*F*	*P*-value
1	4.336	4.451	21.53	3.993	.764	35.71	.00056
2	3.539	5.452	18.18	4.130	.596	10.32	.01481
3	5.564	3.468	32.28	4.666	.821	32.09	.00076
4	3.399	5.677	16.87	3.991	.799	27.91	.00022
5	3.696	5.221	17.77	3.865	.815	48.57	.00284
6	4.794	4.025	18.11	3.038	.742	20.13	.00162
7	4.792	4.027	21.42	3.594	.779	24.72	.00024
8	3.662	5.270	17.97	3.946	.851	63.01	.00008

[a] Cr_a is the asymptotic value of fecal Cr concentration
[b] FO_{CA} is the fecal output, FO_{CA} = dose of Cr/Cr_a
[c] TT is turnover time of the prefecal mass, TT = -1/k
[d] PFM is prefecal mass or mixing compartment, PFM = $FO_{CA} \bullet$ TT

Appendix Table 21. Fecal concentrations (mg/kg DM) of chromium for horses offered orchardgrass/alfalfa hay (Diet 1a) and tall fescue/alfalfa hay (Diet 2a) in Experiment 1[a]

Horse	Diet 1	Diet 2
1	4464.5	3857.6
2	3863.8	4249.8
3	3860.0	4739.4
4	4001.6	3605.6
5	3093.1	4154.7
6	5053.0	4920.0
7	4151.3	4395.3
8	3768.5	4672.9
Mean ± SE[b]	4032 ± 200[c]	4324 ± 159[c]

[a] Fecal samples from total collection for d 5 to d 8 of dosing

[b] Standard error of the mean (n = 8)

[c] (P = .231)

Appendix Table 22. Fecal concentrations (mg/kg DM) of chromium for horses offered orchardgrass/alfalfa hay (Diet 1b) and tall fescue/alfalfa hay (Diet 2b), using fecal grab data in Experiment 1[a]

Horse	Diet 1	Diet 2
1	3889.4	3824.7
2	4342.1	3117.2
3	4542.3	4410.0
4	2971.7	3032.4
5	3402.6	3269.2
6	3509.8	4230.6
7	4684.5	4114.8
8	2999.6	3061.6
Mean ± SE[b]	3792 ± 239[c]	3632 ± 203[c]

[a] Fecal samples from d 1 to d 8 of dosing

[b] Standard error of the mean (n = 8)

[c] (*P* = .451)

Apendix Table 23. Dry matter intake (DMI, kg/d), total fecal output (FO, kg/d), and total collection DMD (D_{TC}, %) for horses offered orchardgrass/alfalfa hay and FF (Diet 3) and orchardgrass/alfalfa hay and SS (Diet 4) in Experiment 2

	Diet 3				Diet 4			
Horse	DMI Hay	DMI Supplement	FO	D_{TC}	DMI Hay	DMI Supplement	FO	D_{TC}
1	5.79	2.32	2.78	64.97	5.82	2.02	2.91	62.86
2	5.91	1.95	2.66	66.10	6.28	2.15	2.86	66.06
3	5.46	1.90	2.74	62.83	5.54	2.02	3.04	59.72
4	4.96	1.58	2.71	58.58	4.97	1.80	2.12	68.66
5	5.81	1.95	3.05	60.78	5.47	1.80	2.82	61.24
6	5.46	1.64	2.31	67.46	4.71	1.89	2.50	62.14
7	4.64	1.64	2.30	63.32	4.71	1.89	2.40	63.69
8	5.42	1.58	2.54	63.75	4.97	1.80	2.26	66.53
Mean ± SE[a]	5.42 ± .15[b]	1.82 ± .09[c]	2.64 ± .08[d]	63.47 ± 1.01[e]	5.31 ± .19[b]	1.92 ± .04[c]	2.61 ± .12[d]	63.86 ± 1.10[e]

[a] Standard error of the mean (n = 8)

[b] (P = .259); [c] (P = .380); [d] (P = .910); [e] (P = .665)

Appendix Table 24. Fecal concentrations (mg/kg DM) of yttrium for horses offered orchardgrass/alfalfa hay and FF (Diet 3) and orchardgrass/alfalfa hay and SS (Diet 4) in Experiment 2[a]

Horse	Diet 3	Diet 4
1	3.25	2.45
2	2.73	3.32
3	2.62	2.17
4	2.22	3.61
5	2.34	3.07
6	2.96	2.51
7	2.85	2.71
8	2.52	3.65
Mean ± SE[b]	2.69 ± .12[c]	2.93 ± .19[c]

[a] Composite fecal samples from d 5 to d 8 of dosing

[b] Standard error of the mean (n = 8)

[c] (P = .414)

Appendix Table 25. Fecal concentrations (mg/kg DM) of ytterbium for horses offered orchardgrass/alfalfa hay and FF (Diet 3) and orchardgrass/alfalfa hay and SS (Diet 4) in Experiment 2[a]

Horse	Diet 3	Diet 4
1	119.3	122.3
2	123.9	112.7
3	126.7	129.2
4	122.5	115.2
5	92.1	105.7
6	136.5	132.1
7	128.1	123.9
8	110.2	111.4
Mean ± SE[b]	119.9 ± 4.2[c]	119.1 ± 3.3[c]

[a] Composite fecal samples from d 5 to d 8 of dosing

[b] Standard error of the mean (n = 8)

[c] (P = .763)

Appendix Table 26. Mean daily fecal concentrations (mg/kg, DM) of chromium for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Cr), and orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Cr) in Experiment 2[a]

Diet 3Cr			Diet 4Cr		
Mean			Mean		
Day	Cr mg/kg DM	SE[b]	Day	Cr mg/kg DM	SE[b]
1	0.0	0.0	1	0.0	0.0
2	0.0	0.0	2	0.0	0.0
3	0.0	0.0	3	0.0	0.0
4	96.8	96.8	4	141.0	107.6
5	4685.0	555.2	5	3453.5	272.8
6	6660.1	1130.0	6	6349.9	429.3
7	6260.8	632.6	7	6362.1	487.3
8	4721.7	221.2	8	4951.3	210.1
9	4171.6	209.5	9	4932.8	289.9
10	4279.8	180.3	10	4569.7	161.4
11	4338.9	273.2	11	4617.8	186.7
12	5393.3	608.8	12	5511.9	321.4
13	1925.3	362.3	13	2533.9	439.7
14	283.0	156.4	14	252.6	134.7
15	0.0	0.0	15	0.0	0.0
16	0.0	0.0	16	0.0	0.0

[a] Daily fecal Cr concentrations used to generate fecal kinetic models
[b] Standard error of the mean (n = 8)

Appendix Table 27. Mean daily fecal concentrations (mg/kg, DM) of ytterbium for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Yb), and orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Yb) in Experiment 2[a]

Diet 3Yb			Diet 4Yb		
Mean			Mean		
Day	Yb mg/kg DM	SE	Day	Yb mg/kg DM	SE
1	0.0	0.0	1	0.0	0.0
2	0.0	0.0	2	0.0	0.0
3	0.0	0.0	3	0.0	0.0
4	6.5	1.5	4	4.5	2.2
5	113.6	10.3	5	94.1	8.0
6	162.9	17.5	6	162.1	7.8
7	165.7	14.6	7	163.2	5.5
8	117.9	7.3	8	134.5	9.0
9	131.0	6.3	9	138.2	9.8
10	131.9	5.0	10	136.2	7.3
11	133.8	4.6	11	134.4	4.1
12	143.4	12.2	12	135.5	6.1
13	52.2	8.1	13	68.2	12.8
14	9.8	2.9	14	12.2	3.9
15	5.35	0.65	15	6.2	0.5
16	0.0	0.0	16	0.0	0.0

[a] Daily fecal Yb concentrations used to generate fecal kinetic models
[b] Standard error of the mean (n = 8)

Appendix Table 28. Individual horse daily fecal concentrations (mg/kg, DM) of chromium for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Cr) in Experiment 2[a]

	Horse							
Day	1	2	3	4	5	6	7	8
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	774.4	0	0
5	4575.4	2563	6259.8	6160	4112.5	6857	3225	3727.5
6	5948	4698.8	11673	4935	4165	10718.5	8214.5	2927.5
7	6406.5	4305	8462	4725	4830	8251	8163	4944
8	4445	4270	5145	4655	4847.5	5512.5	5311.3	3587.5
9	3972.5	5215	4366.3	4252.5	4480	3412.5	4278.8	3395
10	4287.5	4865	4270	4532.5	3745	4235	4891.3	3412.5
11	3088.8	4725	4287.5	4357.5	4025	5521.25	5048.8	3657.5
12	7175	4427.5	6230	3902.5	3001	5880	8032.5	4497.5
13	2325	3150	3220	595	560	1487.5	2320	1745
14	1184	428.75	0	0	0	0	651	0
15	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0

[a] Daily fecal Cr concentrations fitted to main OGFF chromium fecal kinetic model

Appendix Table 29. Individual horse daily fecal concentrations (mg/kg, DM) of ytterbium for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Yb) in Experiment 2[a]

	Horse							
Day	1	2	3	4	5	6	7	8
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	3.5	5.25	7.7	4.9	14	7.35	9.45
5	89.25	82.25	101.15	145.25	126.7	163.45	89.95	110.95
6	147.7	182	201.25	124.95	88.9	249.2	170.1	138.95
7	181.3	161.6	217.35	140	134.1	229.6	150.5	110.95
8	160.95	123.55	101.85	121.8	107.45	120.4	90.65	116.9
9	141.53	142.45	141.05	152.6	137.2	109.9	121.8	101.85
10	112.35	148.75	131.95	141.75	138.25	143.15	128.8	110.25
11	122.5	144.2	148.4	120.05	116.9	149.45	133	135.8
12	202.2	133.7	157.5	120.05	95.55	138.6	178.85	121.1
13	63.35	83.3	70.7	21.35	18.2	45.5	54.25	60.55
14	23.8	15.75	0	4.2	5.6	6.3	17.15	5.6
15	8.05	6.01	7	3.3	3	5.3	6.3	3.8
16	0	0	0	0	0	0	0	0

[a] Daily fecal Yb concentrations fitted to main OGFF ytterbium fecal kinetic model

Appendix Table 30. Individual horse daily fecal concentrations (mg/kg, DM) of chromium for horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Cr) in Experiment 2[a]

	Horse							
Day	1	2	3	4	5	6	7	8
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0.0	856.5	271.3	0	0.0
5	3578.8	3241.0	2327.5	2783.0	4524.0	4515.0	3123.8	3535.0
6	6466.0	6245.8	4865.0	6480.0	7285.3	5040.0	5792.5	8624.6
7	6055.0	5669.0	5600.0	7803.0	6165.0	5652.5	4882.5	9070.0
8	5722.0	5215.0	5372.5	3998.8	4366.3	5092.5	4497.5	5346.3
9	5057.5	3946.3	6107.5	4077.5	4672.5	6055.0	5110.0	4436.3
10	4690.0	4305.0	4987.5	5101.3	3797.5	4935.0	4602.5	4138.8
11	4795.0	3850.0	5451.0	4576.3	4103.8	5162.5	4655.0	4348.8
12	5390.0	4182.5	5075.0	4751.3	6689.0	5827.5	5355.0	6825.0
13	3447.5	1890.0	5083.8	1750.0	1294.0	1933.0	2957.0	1916.0
14	769.5	0	350	0	0	0	901	0
15	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0

[a] Daily fecal Cr concentrations fitted to main OGSS chromium fecal kinetic model

Appendix Table 31. Individual horse daily fecal concentrations (mg/kg, DM) of ytterbium for horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Yb) in Experiment 2[a]

	Horse							
Day	1	2	3	4	5	6	7	8
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	2.8	0.0	18.2	8.4	3.15	3.5
5	91.4	70.7	62.0	97.3	107.1	135.8	87.9	100.8
6	164.9	164.5	136.2	144.2	151.2	164.9	161.7	209.7
7	157.5	155.1	162.1	168.7	186.9	162.1	135.1	178.2
8	145.6	124.3	146.3	156.1	147.6	142.8	137.6	75.6
9	136.9	132.5	161.4	115.9	107.8	176.4	168.0	106.8
10	160.3	118.3	150.2	127.8	115.5	164.5	141.4	112.0
11	144.9	119.4	144.2	139.7	119.0	148.1	129.5	130.9
12	148.1	114.1	149.8	116.6	159.6	142.5	133.7	119.8
13	96.6	41.0	130.9	51.8	27.0	52.2	100.1	46.2
14	25.55	0	13.3	0	11.2	4.9	29.75	12.95
15	7.3	4.55	5.4	8.05	7	3.5	6.7	7
16	0	0	0	0	0	0	0	0

[a] Daily fecal Yb concentrations fitted to main OGSS ytterbium fecal kinetic model

Appendix Table 32.

Fits of data to a one-compartment model of fecal kinetics without a delay, the administration curve with a delay, the post-administration curve, and the total curve with a delay between administration of an oral dose of Cr and its entry into the compartment, in horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement in Experiment 2[a, b, c]

	No Delay			Administration Curve			Post-Administration Curve			Total Curve		
Horse	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value
1	.619	11.35	.0119	.742	20.09	.0029	.612	4.73	.1179	.721	31.05	.0001
2	.894	59.08	.0001	.923	84.44	.0000	.738	8.45	.0621	.910	121.82	.0000
3	.629	11.86	.0108	.750	21.02	.0025	.439	1.57	.3374	.693	24.80	.0004
4	.690	15.59	.0055	.789	26.12	.0014	.787	7.38	.1129	.834	60.40	.0000
5	.814	30.73	.0009	.872	47.48	.0002	.910	20.14	.0462	.878	79.06	.0000
6	.611	10.98	.0129	.715	17.56	.0041	.525	2.21	.2757	.707	26.59	.0003
7	.578	9.59	.0174	.702	16.52	.0048	.621	4.91	.1570	.704	28.52	.0002
8	.748	20.74	.0026	.824	32.82	.0007	.723	5.22	.1498	.842	63.86	.0000

[a] Individual horse data
[b] Delay from mean OGFF model (6.792 h)
[c] Model fitted using the chromium external marker

Appendix Table 33.

Fits of data to a one-compartment model of fecal kinetics without a delay, the administration curve with a delay, the post-administration curve, and the total curve with a delay between administration of an oral dose of Yb and its entry into the compartment, in horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement in Experiment 2[a, b, c]

	No Delay			Administration Curve			Post-Administration Curve			Total Curve		
Horse	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value	R^2	*F*	*P*-value
1	.738	19.77	.0030	.816	31.05	.0008	.650	7.43	.0527	.778	45.61	.0000
2	.848	39.09	.0004	.852	40.24	.0004	.845	21.85	.0095	.858	78.41	.0000
3	.624	11.63	.0113	.731	19.00	.0033	.790	15.03	.0179	.751	39.15	.0000
4	.831	34.54	.0006	.875	48.84	.0002	.871	26.98	.0065	.902	120.04	.0000
5	.818	31.39	.0008	.860	42.89	.0003	.942	65.13	.0013	.875	91.05	.0000
6	.573	9.39	.0182	.688	15.43	.0057	.839	20.87	.0103	.732	35.47	.0001
7	.679	14.83	.0063	.775	24.16	.0017	.719	10.21	.0331	.767	42.78	.0000
8	.847	38.70	.0004	.883	52.67	.0002	.885	30.82	.0052	.893	107.99	.0000

[a] Individual horse data
[b] Delay from mean OGFF model (5.520 h)
[c] Model fitted using the ytterbium external marker

Appendix Table 34. Estimates of fit of individual fecal ytterbium concentration data to a one-compartment model, model parameters, and calculated variables in horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Yb) in Experiment 2

Horse	Cr_a, mg/kg[a]	FO_{CA}, kg/d[b]	TT, h[c]	PFM, kg[d]	R^2	*F*	*P*-value
1	.148	4.514	26.34	4.954	.816	31.05	.00084
2	.150	4.448	29.75	5.514	.858	78.41	.00005
3	.155	4.313	27.05	4.863	.751	39.15	.00042
4	.143	4.655	24.50	4.753	.902	120.04	.00001
5	.135	4.957	24.11	4.980	.875	91.05	.00003
6	.164	4.073	23.77	4.034	.732	35.47	.00057
7	.145	4.610	25.11	4.823	.767	42.78	.00032
8	.130	5.128	26.18	5.594	.893	107.99	.00002

[a] Cr_a is the asymptotic value of fecal Cr concentration
[b] FO_{CA} is the fecal output, FO_{CA} = dose of Yb/Cr_a
[c] TT is turnover time of the prefecal mass, TT = -1/k
[d] PFM is prefecal mass or mixing compartment, PFM = $FO_{CA} \bullet$ TT

Appendix Table 35. Estimates of fit of individual fecal ytterbium concentration data to a one-compartment model, model parameters, and calculated variables in horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Yb) in Experiment 2

Horse	Cr_a, mg/kg[a]	FO_{CA}, kg/d[b]	TT, h[c]	PFM, kg[d]	R^2	*F*	*P*-value
1	.151	4.435	28.45	5.258	.802	52.65	.00017
2	.143	4.664	23.84	4.633	.871	87.80	.00003
3	.157	4.253	24.47	4.337	.877	49.99	.00020
4	.150	4.462	23.51	4.370	.819	58.70	.00012
5	.145	4.616	25.75	4.953	.848	72.32	.00006
6	.163	4.095	26.04	4.443	.883	97.93	.00002
7	.152	4.400	32.61	5.980	.857	77.76	.00005
8	.152	4.381	23.23	4.242	.811	55.90	.00014

[a] Cr_a is the asymptotic value of fecal Cr concentration
[b] FO_{CA} is the fecal output, FO_{CA} = dose of Yb/Cr_a
[c] TT is turnover time of the prefecal mass, TT = -1/k
[d] PFM is prefecal mass or mixing compartment, PFM = FO_{CA} • TT

Appendix Table 36. Fecal concentrations (mg/kg DM) of chromium for horses offered orchardgrass/alfalfa hay and FF (Diet 3) and orchardgrass/alfalfa hay and SS (Diet 4) in Experiment 2

Horse	Diet 3	Diet 4
1	4090.5	4545.5
2	3830.2	4059.1
3	5557.9	4338.8
4	4202.2	4352.5
5	3775.6	4471.4
6	5660.3	4590.5
7	4891.5	4082.9
8	3206.4	4937.5
Mean ± SE[a]	4401 ± 311[b]	4422 ± 100[b]

[a] Standard error of the mean (n = 8)

[b] (*P* = .955)

Appendix Table 37. Fecal concentrations (mg/kg DM) of ytterbium for horses offered orchardgrass/alfalfa hay and FF (Diet 3) and orchardgrass/alfalfa hay and SS (Diet 4) in Experiment 2[a]

Horse	Diet 3	Diet 4
1	119.4	125.2
2	123.5	110.5
3	131.1	120.6
4	119.3	118.7
5	106.8	119.2
6	147.4	137.8
7	111.5	120.5
8	104.4	114.7
Mean ± SE[a]	120.4 ± 4.9[b]	120.9 ± 2.8[b]

[a] Individual samples d1 to d 8 of dosing

[b] Standard error of the mean (n = 8)

[c] (*P* = .894)

Appendix Table 38. Diurnal variation in fecal concentrations (mg/kg, DM) of chromium for individual horse fecal grab samples collected on d 8 of dosing, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Cr) in Experiment 2

	Diurnal Fecal Grab Samples[a]				
Horse	0700	1500	2300	Mean Diurnal	Mixed[b]
1	4883.5	2616.0	5444.0	4314.5	3088.8
2	5915.0	6667.5	6597.5	6393.3	4725.0
3	5549.3	5254.4	5280.0	5361.2	4287.5
4	5468.8	4357.5	4270.0	4698.7	4357.5
5	4182.5	4584.0	5057.5	4608.1	4025.0
6	5534.4	5927.3	8954.8	6805.4	5521.3
7	5865.0	6524.9	8053.0	6814.3	5048.8
8	5040.0	4655.0	4042.5	4579.2	3697.5
Mean ± SE[c]	5304 ± 204[d]	5073 ± 470[d]	5962 ± 624[d]	5446 ± 375[e]	4343 ± 271[e]

[a] Fecal grab samples collected at 0700, 1500 and 2300
[b] Fecal grab samples collected at 0700, 1500 and 2300, and combined
[c] Standard error of the mean (n = 8)
[d] Coefficient of variation (8.47 %)
[e] (P = .0004)

Appendix Table 39. Diurnal variation in fecal concentrations (mg/kg, DM) of chromium for individual horse fecal grab samples collected on d 8 of dosing, for horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Cr) in Experiment 2

	Diurnal Fecal Grab Samples[a]				
Horse	0700	1500	2300	Mean Diurnal	Mixed[b]
1	7656.0	7848.0	4156.0	6553.3	4795.0
2	4686.5	4979.0	5969.0	5211.5	3850.0
3	4217.5	7455.0	6965.0	6212.5	5451.0
4	5826.5	4331.0	5494.0	5217.1	4576.3
5	5762.0	3716.0	4285.5	4587.8	4103.8
6	7700.0	7455.0	4077.5	6410.8	5162.5
7	5810.0	6195.0	5897.5	5967.5	4655.0
8	5461.0	4626.0	5120.0	5069.1	4348.8
Mean ± SE[c]	5889 ± 440[d]	5825 ± 573[d]	5245 ± 364[d]	5653 ± 255[e]	4617 ± 187[e]

[a] Fecal grab samples collected at 0700, 1500 and 2300
[b] Fecal grab samples collected at 0700, 1500 and 2300, and combined
[c] Standard error of the mean (n = 8)
[d] Coefficient of variation (6.27 %)
[e] (P = .0003)

Appendix Table 40. Diurnal variation in fecal concentrations (mg/kg, DM) of ytterbium for individual horse fecal grab samples collected on d 8 of dosing, for horses offered orchardgrass/alfalfa hay and fat-and-fiber supplement (Diet 3Yb) in Experiment 2

	Diurnal Fecal Grab Samples[a]				
Horse	0700	1500	2300	Mean Diurnal	Mixed[b]
1	187.3	136.2	136.5	153.3	122.5
2	158.9	200.6	185.5	181.6	144.2
3	232.1	235.2	137.2	201.4	148.4
4	172.2	117.6	122.5	137.4	120.1
5	137.9	128.5	125.3	130.5	116.9
6	213.9	162.8	206.5	194.3	149.5
7	169.1	178.5	168.0	171.8	133.0
8	200.9	167.3	135.5	167.8	135.8
Mean ± SE[c]	184 ± 10[d]	165 ± 13[d]	152 ± 10[d]	167 ± 9[e]	134 ± 4[e]

[a] Fecal grab samples collected at 0700, 1500 and 2300
[b] Fecal grab samples collected at 0700, 1500 and 2300, and combined
[c] Standard error of the mean (n = 8)
[d] Coefficient of variation (9.56 %)
[e] (P = .0002)

Appendix Table 41. Diurnal variation in fecal concentrations (mg/kg, DM) of ytterbium for individual horse fecal grab samples collected on d 8 of dosing, for horses offered orchardgrass/alfalfa hay and sugar-and-starch supplement (Diet 4Yb) in Experiment 2

	Diurnal Fecal Grab Samples[a]				
Horse	0700	1500	2300	Mean Diurnal	Mixed[b]
1	217.7	191.5	153.3	187.4	144.9
2	174.3	138.6	145.6	152.8	119.4
3	158.2	182.7	175.7	172.2	120.1
4	239.8	151.2	142.1	177.6	139.7
5	147.7	161.7	127.1	145.4	119.0
6	232.8	202.7	116.6	183.9	148.1
7	170.1	153.3	164.9	162.7	129.5
8	185.2	140.0	134.1	153.1	130.9
Mean ± SE[c]	191 ± 12[d]	165 ± 8[d]	145 ± 6[d]	167 ± 5[e]	131 ± 4[e]

[a] Fecal grab samples collected at 0700, 1500 and 2300
[b] Fecal grab samples collected at 0700, 1500 and 2300, and combined
[c] Standard error of the mean (n = 8)
[d] Coefficient of variation (8.08 %)
[e] (*P* = .0001)

Vita

Belinda Jane Hargreaves, daughter of Mrs. Jane Jones and Mr. Paul Hargreaves, was born on December 11, 1961 in Plymouth, Devon, U. K. She graduated from Uffculme School, Devon, U. K., in 1978, and attended East Devon College studying business and commerce for one year. During the following 13 years she was a professional rider, instructor and trainer in Europe and America. She attended the University of Bristol, U. K. in 1992, majoring in Biology and Geography (an interdisciplinary program in environmental science), and received her Bachelor of Science Joint-Honors degree in July 1996. In 1997 she was awarded a WALTHAM Fellowship to pursue a Masters degree in Equine Nutrition in the Department of Animal and Poultry Sciences at Virginia Polytechnic Institute and State University. She was awarded a research grant by the Virginia Horse Industry Board to study vitamin E supplementation of grazing mares. In 1998 she was awarded a John L. Pratt Fellowship to pursue a doctoral degree in Equine Nutrition and Exercise Physiology from the Department of Animal and Poultry Sciences at Virginia Polytechnic Institute and State University.

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