Alkanes as Internal and External Markers in Horses and the Digestibility of a High Fat Cereal By-Product

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(ABSTRACT)

Determining intake of feeds in horses is an important factor in incorporating supplements in their diets. Fecal recoveries (R), fecal output (FO), dry matter digestibility (DMD) and dry matter intake (DMI) were estimated using alkanes as markers in 8 thoroughbred geldings. The experiment compared two diets in a 2 X 2 latin square experiment. The diets were mixed grass hay only (H) and the same hay plus a cereal by-product (H + CBP). The cereal by-product (CBP) was the high fat component added to feeds at Virginia Tech's Middleburg Agricultural Research and Extension Center. The apparent digestibility of ether extract (EE) and other nutrients in the H and H + CBP, as well as the partial digestibility of CBP were also determined. The periods were 21 d each with a dietary accommodation period followed by eight days of dosing the even chain alkanes dotriacontaine (C32) and hexatriacontane (C36) as external markers. Total collection (TC) was performed the last 4 d of dosing.

The results show that mean recoveries of alkanes were close to 100%, but the range for individual alkanes was wide, and the pattern of recoveries for alkanes of different

Abstract

chain length was inconsistent from feed to feed. The results also indicate that mean estimates of the DMI, DMD and FO of a feed, such as H or H + CBP, are determined with reasonable accuracy by means of alkane markers. In contrast, alkane estimates of DMI and DMD in an individual horse fail to predict corresponding TC estimates. The alkane estimate of FO in an individual horse predicts a TC value with error of 16.4%. The CBP was found to be an excellent source of EE, CP and fiber but a poor source of Ca.

(Keywords: alkanes, markers, digestibility, fecal output, dry matter intake, and horse)

Abstract

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Table of Contents

List of Tables

Table **Page**

List of Figures

Figure **Page**

Appendix

Appendix Table **Page**

Introduction

Alkanes. As grazing herbivores, horses are able to meet all of their nutritional requirements from quality forage. Horses with increased nutritional demands due to exercise, growth, lactation and reproduction require the addition of a feed supplements, or concentrates. Measurements of dry matter digestibility (DMD), fecal output (FO) and dry matter intake (DMI), are required for ascertaining the complete nutrition of grazing animals, and determining the need to incorporate supplemental feeds.

Direct measurements of DMD, FO and DMI are difficult in the field; therefore, nutritionists have employed external and internal marker substances to estimate these variables (Kotb and Luckey, 1972; Mayes et al., 1995). Internal markers are substances that either exist naturally in the feed or are produced from the feed. External markers are substances that are added to the feed (Mayes et al., 1995). Markers should be chemically discrete, indigestible and unabsorbable and should pass through the digestive tract of the animal at a constant rate. Markers should also be similar physically to the material that they are to mark (Kotb and Luckey, 1972; Mayes et al., 1995).

In the mid 1980's, alkanes were introduced as potential markers in sheep (Mayes and Lamb, 1984). These chemically discrete compounds are long-chain saturated hydrocarbons that exist in the cuticular wax of most plants. The alkanes that are found in plants are odd- chained and can be used as internal markers to estimate DMD (Mayes and Lamb, 1984). When dosed to the animal artificially, even-chain alkanes can be used as external markers to estimate FO. Combining them estimates DMI.

Intake of feed (DMI) in grazing horses can be estimated using an internal marker to measure DMD and an external marker to measure FO. Mayes et al. (1986a) first developed a double alkane method for estimating intake in sheep. In this method, animals are dosed with known amounts of the even-chain alkane C32. Intake is then calculated from the daily dose rate and the fecal and dietary concentrations of the even-chain alkane and an adjacent odd-chain alkane that is inherent in the diet (Mayes et al., 1986a; Mayes et al., 1986b; Mayes et al., 1995).

Few studies have been reported using alkanes as markers in horses. Ordakowski et al (2001) examined the use of odd-chain alkanes as internal markers to estimate DMD of hay and hay plus concentrate diets in horses. McMeniman et al. (1990) examined even-chain alkanes as external markers to predict FO. A subsequent study used alkanes to estimate DMI in horses, although the results of this study are not published (Nash, 2001).

Digestibility. Fat, or ether extract (EE), is added to the diets of horses to increase energy density for performance and to avoid adverse effects of high starch and sugar equine grains. The fermentative equine grain-associated disorders including, gastric ulcers, osmotic diarrhea, distention colic, acidic colic, laminitis and founder may be avoided by feeding high fat diets. The metabolic disorders that may be avoided are extertional rhabdomyolysis (tying-up) and ostiochondrosis. Dietary fats are also needed for the absorption of fat-soluble vitamins, which may become inadequate from low fat feeds.

Despite that horses evolved as herbivores, consuming feed low in fat, they can digest fat when comprises up to 20% of their diet. When the amount of fat is kept below this 20% level, digestibility is over 85% (Kane et al., 1979; Snyder et al., 1981). The

digestibility by horses of the EE component in feeds varies greatly from forages to grains and further to added fats consisting mainly of triglycerides (Bowman et al., 1979; Kane et al., 1979; Hintz and Schryver, 1989; Sturgeon et al., 2000). The digestibility of EE in forages is lower than grains due to the presence of waxes, sterols, pigments and other indigestible lipids (VanSoest, 1984).

In palatability comparison tests of fats, corn oil has had the highest acceptance by horses and tallow the lowest (Holland et al., 1998a). According to common experience, the high fat cereal byproduct fed at the Middleburg Agricultural Research and Extension Center is also highly palatable. Fats should be incorporated into feeds and balanced to meet current recommendations on a digestible energy (DE) basis. Adding fats to already complete and balanced feed may result in deficiencies of essential nutrients, with attention given to protein, Ca, Mg, and antioxidant (Kronfeld et al., 2001). Associative effects of a high fat supplement can be evaluated through the determination of partial digestibility of the added supplement.

The main objectives of this study were to validate the use of alkanes as internal and external markers to estimate DMD, FO and DMI for a group of horses, and for individual horses, offered hay only (H) or hay plus cereal by-product (H + CBP). A secondary objective was to determine the partial digestibility of EE and other nutrients in the CBP supplement.

Review of Literature

Alkanes

Indirect methods of determining dry matter intake (DMI), dry matter digestibility (DMD) and fecal output (FO) are needed to evaluate nutrition of horses and formulate optimal forage supplements. The nutritional demands of most horses at maintenance are met through the consumption of quality pasture. Performance horses, growing horses and those enduring reproductive stresses have increased nutritional demands that cannot be met by pasture alone. For horses with elevated nutritional needs, forage supplements are incorporated into their ration, usually in the form of hay and concentrates. Pasture DMI and overall DMD should be determined, as well as the supplements effect on intake and digestibility, in order to properly incorporate supplements into the diet.

Estimates of DMI, DMD and FO for livestock are traditionally determined in digestion trials. Conventional digestibility trials use total collection (TC) measurements to estimate FO and DMD (VanDyne, 1969; Sutton et al., 1977; Holechek et al., 1986). In TC experiments, feeds offered and refused feeds (orts) are weighed to determine DMI. The FO is determined by total collection of feces, either manually or by a collection tray or harness. From this, apparent DMD can be determined by assuming that DMD of forage is the difference between DMI and FO (Minson, 1990). The TC method is effective for confined animals and usually serves as the standard to validate other methods used to estimate DMI, DMD and FO. However, TC is labor intensive and expensive, which restricts the number of animals to be studied. There are also errors associated with DMD derived from the TC method. Stall confinement and fecal collection harnesses may disrupt the natural grazing behavior of animals, thus altering

DMD,FO and DMI estimates. In addition, incomplete total collection of orts and feces results in an overestimation of DMD.

The "by difference" method is routinely used for estimating DMI of pasture (Pagan, 1995; Kronfeld, 1998). With this method, estimates of mean digestible energy (DE) of supplements are subtracted from the National Research Council (NRC, 1989) estimates of mean DE intake, which are based on body weight and physiological condition of the horse. Any difference in DE is assumed to be provided by the DMI of pasture by the horse. Since errors are large and may exceed 100%, negative DMI values have often been calculated using this method. These large errors have prompted the development of alternative methods of estimating DMI.

Digestibility Markers

Direct measurements of DMI, DMD and FO are difficult to measure in the field; therefore nutritionists estimate these variables indirectly by employing markers (Mayes et al., 1995). When validated and used correctly, digestibility markers provide a reliable method for estimating DMI in grazing livestock (Kotb and Luckey, 1972). A marker must be indigestible, unabsorbable and physically inert. Markers must be easy to analyze and pass through the digestive system at a steady rate (Maynard et al., 1979). A marker that is either an inherent part of feed or forage, or produced from the feed or forage is called an internal marker. External markers are separate from the feed and are artificially administered or dosed to the animal (Mayes et al., 1995).

Internal and external Markers can be used to estimate DMD, DMI and FO. The following equation shows the indigestibility of a marker where DMI is the daily dry matter intake of food, C_I is the concentration of the marker in the feed, FO is fecal output and C_F is the concentration of the marker in the feces (Kleiber, 1961):

$$\mathbf{DMI * C_I = FO * C_F \text{ or}}$$
$$\mathbf{C_I/C_F = FO/DMI}$$

Dry matter digestibility (DMD) is estimated with the following equation (Kleiber, 1961):

$$\mathbf{DMD, \% = [(1 - FO)/DMI]*100 = (1 - FO/DMI)*100}$$

When markers are employed, C_I and C_F are the concentrations of an internal marker in the feed and feces respectively; DMD can be estimated with the following equation:

$$\mathbf{DMD, \% = (1 - C_I /C_F)*100}$$

The FO is determined with the use of external markers. The marker must be given daily until it reaches a plateau level in the feces, which is usually a period of 4 to 5 d (Pond et al., 1987). Thus, FO can be calculated by the following equation, where **M** represents the amount of the external marker dosed and the concentration of the external marker in the feces is **[M]**:

$$\mathbf{FO\ (kg/d) = M\ dosed\ (g/d) / [M]\ feces\ (g/kg)}$$

The estimated FO can then be used to determine DMI by calculating the following:

$$\mathbf{DMI\ (kg/d) = FO * (C_F/C_I)}$$

Markers in Animal Digestion Studies

The principle of using markers was first proposed by Wildt (1874) using silica. Many indigestible substances studied as markers have not been discrete chemical entities; therefore what is measured in the feces does not equal what was fed (Mayes et al., 1995). As a result, some markers such as lignin and indigestible acid detergent fiber have given poor results (Dove and Coombe, 1992; Cochran et al., 1986). Other methods were developed to overcome these inadequacies, one being *in vitro* incubation methods.

The *in vitro* method provides DMD estimates based on forage samples of known *in vivo* DMD (Minson, 1990). Forage samples are ground and undergo fermentation with microorganisms in a buffered medium, usually rumen liquor, under controlled conditions of anaerobiosis, temperature and pH (Tilley and Terry, 1963; Minson, 1990). *In vivo* DMD estimates correlated with *in vitro* DMD estimates in a 48 h digestion trial with rumen fluid, followed by a 24 h digestion with pepsin-HCL (Tilley and Terry, 1963).

Trevor-Jones et al. (1991) adapted this method for determining the DMD of equine feeds. In their study the cecal fluid from horses, in place of rumen liquor, was used to estimate the DMD of feed. There are several disadvantages to the *in vitro* method. The technique assumes that all individual animals in the study will have the same digestive efficiency and that the digestibility of forage is unaffected by the level of intake or the feeding of protein or supplements high in starch (Orton et al., 1985; Martin et al., 1989).

The disadvantages of *in vitro* digestibility methods and technological advances in quantifying discrete compounds in forage and feces has led to the need for new and improved internal markers to determine digestibility.

Internal Markers in Animal Digestion Studies

Several internal markers have been used in animal digestion studies including lignin, acid insoluble ash, rare earths, and plant fibers such as indigestible acid detergent and neutral detergent fiber. Lignin was one of the first plant components used as an internal marker, dating back to the late 1800's (Kotb and Lucky, 1972). Lignin is often regarded as being indigestible because there appear to be no microbial or mammalian enzymes capable of lignin degradation (Van Soest, 1982). However, studies indicated that there were problems with the fecal recovery and quantification of lignin. Lignin underwent some digestion by fungi. In addition, it is difficult to analyze (Elam and Davis, 1961). Schurg (1981) investigated lignin as an internal marker in horses and found that it underestimated DMD by 12 to 18%, concluding lignin is an unreliable internal marker.

A commonly used internal marker employed to estimate DMD in horses is acid insoluble ash (AIA). The DMD estimates using AIA as an internal marker were similar to total collection DMD in horses (Shurg, 1981). However, digestibility was overestimated when AIA was used in ponies, horses and white rhinoceros (Frape, 1982). In studies with stall-fed horses, DMD was consistently underestimated using AIA when compared to total collection DMD, yet differences were not significant (Sutton et al. 1977; Orton et al., 1985; Cuddeford and Hughes, 1990).

Rare earth elements have also been used as internal markers to estimate DMD. Sm and La are not successful as markers when applied to soybean and cottonseed meal, as well as ground corn because they dissociate from feeds and have a higher rate of passage (Crooker et al., 1982). Subsequent research using Yb, Er, Dy, Sa and Y found minimal detachment from the feed when applied to different sized corn particles (Turnball and Thomas, 1987). Rare earth elements were also found to be affected by time of feeding (Pond et al., 1989).

Methods using acid detergent fiber (ADF) and neutral detergent fiber (NDF) have shown limited success as internal markers. When used in sheep, ADF predicted the DMD of several forages in sheep, yet it was mentioned that more plant species needed to be examined (Penning and Johnson, 1983a; Penning and Johnson 1983b). A study by Meacham (1987) showed that NDF could be used to estimate apparent DMD of tall fescue by horses. A similar study found that NDF accurately estimated the apparent DMD of tall fescue and orchardgrass/clover pasture grazed by young light breed horses (Moffit et al., 1987). The previous two studies did not compare NDF estimates to total collection; therefore, validation studies need to be conducted to justify the use of NDF as an internal marker.

Grace and Body (1981) discovered that long-chain fatty acids of chain length C19-C32, which are present in plant cuticular wax, were recovered in the feces of sheep, and can be used as internal markers. More recently, there has been a considerable amount of work done with other plant-wax compounds as markers, specifically the hydrocarbons.

Estimation of Fecal Output Using External Markers

Fecal output (FO) can be measured directly in grazing animals by total collection using bags attached to the animals. Since this method is tedious and may disturb the natural foraging behavior of animals, external markers are orally dosed to estimate FO and then compared to total collection measurements. Chromic oxide (Cr_2O_3) is used most frequently as an external marker in nutrient digestion studies because it is relatively easy to analyze, it is inexpensive, and it can be readily incorporated into the animals' feed (Haenlein et al. 1962; Schurg, 1981; Barbisan et al., 1993;Titgemeyer, 1997; Holland et al., 1998b). Once Cr_2O_3 fecal concentrations reach equilibrium, after 5 to 6 d, fecal samples can be collected off the ground or from rectal grab samples (Mayes et al., 1995). One of the problems with the technique is diurnal variation in Cr_2O_3 excretion rates (Haenlin et al., 1966; Cuddeford and Hughes, 1990; Holland et al., 1998b). This problem may be overcome by dosing via a continuous release bolus (Luginbuhl et al., 1994). Chromic oxide passage rates have also been shown to decrease with increasing ingesta particle size (Bruining and Bosch, 1992). Yterrbium (Yb), a rare earth element, has also shown some success as an external marker for grazing horses (Meacham, 1987; Moffit et al., 1987, Martin et al., 1989). However, these studies did not validate Yb's estimates of FO with TC measurements.

Using Double Markers in Equine Digestion Studies

Determining DMI in grazing horses can be estimated using an internal marker and an external marker together; thus double markers. Cr_2O_3 and AIA were used to predict pasture intake in pregnant and lactating mares (Martin et al., 1989). When compared to the NRC (1989), predicted digestible energy (DE) intakes were 5 to 20% lower than expected. Cr_2O_3 and AIA were compared to Cr_2O_3 and permanganate lignin as double markers to predict nutrient DMD in horses (Schurg, 1981). While lignin consistently underestimated DMD by 10 to 20%, Cr_2O_3 and AIA were within 4% of the measurements from total collection (Martin et al., 1989).

Alkanes as Markers

Grace and Body (1981) discovered that long-chain fatty acids of chain length C19-C32, which are present in plant cuticular wax, were recovered in the feces of sheep, and can be used as internal markers. More recently, there has been a considerable amount of work done with other plant-wax compounds as markers, specifically the hydrocarbons. Hydrocarbons appear to be omnipresent in the cuticular wax of higher plants. In most plant species, the predominant hydrocarbons are alkanes ranging in chain length from 21 to 37 carbon atoms (i.e. C21 to C37). Over 90% of the alkanes found in plants have odd numbers of carbon atoms (Mayes et al., 1995). Nonacosane (C29), hentriacontane (C31) and tritriacontane (C33) typically have the highest concentrations in herbage (Dove and Mayes, 1991). Alkanes can be easily analyzed using gas chromatography and their

inertness are the primary reasons for considering the use of alkanes as internal markers (Mayes and Lamb, 1984). Only 3 to 6% of the alkanes found in plant waxes are even-chained, therefore these compounds can be used as external markers to predict fecal output (Dove and Mayes 1991). Double marker methods can be applied when even and odd-chain alkanes are paired together, assuming they have similar recoveries (i.e. C32 and C33) (Mayes et al., 1986a).

Alkanes as Internal Markers to Estimate Digestibility

Odd-chain alkanes from C25 to C33 have been examined as internal markers in horses (Ordakowski et al. 2001; O'Keefe and McMeniman, 1998), sheep and cattle (Mayes and Lamb, 1984; Dove et al., 1999; Unal and Garnworthy, 1999), and exotic animals (Hatt et al. 1998; Gedvir and Hudson, 2000). There have been several studies with sheep (Mayes et al., 1986a; Dove and Mayes, 1991; Vulich et al., 1991), goats (Mayes et al., 1995) and cattle (Dillon and Stakelum, 1995) in which fecal recoveries were quantified. In many studies, recoveries increased with increasing alkane chain length. Because of incomplete recovery of alkanes in the feces, DMD estimates depend on the degree to which the recovery values vary. In studies with ruminants (Mayes et al., 1986a; Mayes et al. 1986b) correction for incomplete recoveries would be needed in order to assume the DMD predictions from alkane were not underestimated. The incomplete recoveries of alkanes may be associated with the complex stomach of the ruminant, as recoveries have been similar among chain lengths in studies with non-ruminants (O'Keefe and McMeniman, 1998; Ordakowski et al., 2001).

Alkanes were used in a study with mature thoroughbred geldings to determine marker recovery, and DMD of four different diets in stalls (Ordakowski et al., 2001). Fecal recoveries of alkanes were not related to chain lengths. C31 and C29 were the most abundant n-alkanes, followed by C33, C27 and C25. This pattern concurs with other work done with grass and legume forage species (Malossini et al., 1990; Dove and Mayes, 1991). For all of the diets estimates of DMD based on individual alkanes were similar to total collection measurements (Ordakowski et al., 2001).

Alkanes as External Markers to Estimate Fecal Output

The synthetic alkanes octacosane (C28), dotriacontane (C32) and hexatriacontane (C36) are most commonly used to estimate FO (Mayes et al. 1986a; Mayes et al., 1986b; Mayes et al., 1988; Hatt et al., 1998; Unal and Garnsworthy, 1999). However, C24 and C26 have also been used as external markers (Duncan et al., 1999). The even-chain alkanes C28 and C36 have been less satisfactory than C32 as dosed markers in double marker methods, although C36 can still be useful in estimating FO since it has a relatively high recovery rate of approximately 95% in ruminants (Mayes et al., 1986b).

Fecal output has been accurately estimated in dairy cows with the administration of C32. Animals were dosed twice daily with paper pellets containing the even-chain alkane to estimate FO to later determine intake (Hameleers and Mayes, 1997). Accurate estimates of FO are used in intake calculations via the double marker method.

Alkanes as Double Markers to Predict Intake

Mayes et al. (1986) first developed a double alkane method for estimating intake. The predominance of odd-chain alkanes in forage species and relatively low cost of even-chain alkanes of similar chain length, together, led to the development of a double marker technique for estimation of intake of feeds and forage. In this method, animals are dosed with known amounts of an even-chain alkane. Intake is then calculated from the daily dose rate and the fecal and dietary concentrations of the even-chain alkane and an adjacent odd-chain alkane that is inherent in the diet (Mayes et al., 1986a; Mayes et al., 1986b). The presence of the even chain alkane in the diet is accommodated in the intake calculation. A study that aimed to estimate pasture intake using the double marker method in weanling thoroughbreds found their success was relative to season. When the pastures were in a reproductive state, the alkane intake estimates were inaccurate because the alkane content in the seed heads is different from the leaf portion of the plants. The study reported high intake estimates when C32 was under dosed (Nash, 2001). This study did not compare alkane estimates with total collection, therefore a further validation would need to be performed to determine the success of alkanes as markers to estimate DMI.

Incomplete fecal recoveries do not affect intake estimates, provided the double markers have similar recoveries. Diurnal variation of the dosed even-chain alkane has been a concern in using this double marker method with alkanes. Diurnal variation in the fecal concentration of dosed alkanes was found to be small when sheep were dosed once per day or twice daily with two different dosing methods (Dillon, 1993). This variation

has been found to be higher in cattle (Dove and Mayes, 1991). However, the ratio of the fecal concentration of the alkane pair used in calculating intake is less prone to temporal variation than are absolute concentrations (Dove et al., 1991).

Future Uses of Alkanes

Alkane Double Marker Estimates of Pasture and Supplement Intake. Indoor stall trials with ruminants have shown that the double marker method with the application of alkanes is reliable (Mayes et al., 1986a; Vulich et al, 1991). However, validation of the method with grazing animals is more difficult as there is no other successful method to which to compare.

There is potential for the double marker method with alkanes to estimate individual DMI of both pasture and supplements when horses are fed in groups. If the methods were successful, studies could be performed with group fed horses with individual horses as the experimental unit, increasing the degrees of freedom, thus increasing the statistical power of the experiment. Concentrations of two or more alkanes in the pasture, supplement and feces are required. Simultaneous equations using two alkanes for supplement and pasture would be used to estimate DMI.

A factor that may affect reliability of the technique under natural grazing situations is variations in daily feeding pattern (Dove and Mayes, 1995). Resultant errors from such effects were small in dairy cows (Dillon and Stakelum, 1993). Another factor that may affect pasture intake estimates is the collection of a representative pasture sample. The diet consumed in the experiment must be accurately sampled in terms of alkane content

in the forage. Pastures that are uniformly sown are relatively easy to sample by hand collection (Vulich et al., 1993). When animals consume complex vegetation communities, collecting samples that accurately reflect what the animal consumed may be difficult.

Determining Diet Composition With Alkanes. Knowledge of what species are consumed is important in that different forage species have varying nutritive value to the horse. Different plant species have characteristic differences in alkane concentration patterns (Mallosini et al., 1990; Laredo et al., 1991; Mayes et al; 1995). Simultaneous equations are used to estimate botanical composition of forages using alkanes as markers (Dove and Coombe, 1992). In this method the number of equations, the number of forage species and the number of alkanes chosen are equal (Dove and Coombe, 1992). Several studies and reviews have validated this method for estimating botanical composition of pasture consumed by animals (Dove and Coombe, 1992; Newman et al. 1995; Dove and Mayes, 1996).

Conclusion

Studies have reported accurate estimates of DMD, FO and DMI using alkanes as internal and external markers in several species of animals. Future uses of alkanes as markers in studies with horses may include the determination of intake of pasture and supplement and the botanical composition of forage intake.

Digestibility

Horses with additional nutritional requirements due to exercise, reproduction and growth require supplemental concentrates in their diets. The advantages and disadvantages of adding food supplements can be determined by the partial digestibility (D_P) of the concentrate, as the addition of supplements may be different from apparent digestibility (D_A). The primary fuel in concentrates is carbohydrates, fat and protein. Added fat to the equine diet may benefit several aspects of equine nutrition (Scott et al., 1989; Potter et al.1992b; Holland et al., 1996a).

Partial Digestibility

An understanding of the nutrient digestion and utilization is necessary in evaluating supplements. Determining the D_A and D_P of nutrients facilitates the development of appropriate supplements. D_A takes into account both the unabsorbed feed residues and the endogenous components of the feces. D_A is measured by how much of a food constituent is fed and how much of the respective constituent is excreted in the feces. The D_A is usually expressed as a percentage (Kleiber et al., 1961).

Partial digestibility (D_P) is used for feed ingredients or supplements that are added to a basal feed, and that cannot be fed exclusively as the whole ration. To determine D_P animals are fed in two feeding trials where the amount of digestible nutrient in the ration with and without the concentrate is determined. The difference in digestible nutrient between the two rations divided by the difference in total nutrients is the D_P. Kleiber

(1961) determined the D_P of two rations when he fed cows two different amounts of Sudangrass hay. Kleiber (1961) showed that it is possible for the D_P of a given feed to differ from its D_A. The addition of a high fat supplement may have associative effects on the digestibility of nutrients in the basal feed.

Fat in Equine Diets

Added fat to horse diets benefits the horse in many aspects. Fats increase energy density, improve performance and decrease adverse effects of excessive sugars and starch common in commercial horse feeds. Although they evolved as herbivores, horses can digest and utilize fat with great efficiency when it comprises up to 20% of the total diet. If less than 20% of the total diet is made up of fat, digestibility of fats and oils is usually over 85%. Palatability tests have shown that vegetable fats are highly accepted by horses and have a higher digestibility (approximately 90%) than fat from animal sources (approximately 75%) (Kane et al., 1979; Snyder et al., 1981; Holland et al., 1998a).

Fat Adaptation

Fats must be added to the diet gradually. When fats are introduced to the diet too quickly, stool may become shiny, then loose, then steatorrhea may occur (Kronfeld et al., 2001). These undesired effects can be avoided by adding fats or fat-fortified feeds in gradually increasing amounts over a period of 1 to 4 days until the desire amount is attained.

Of all the chemical forms of dietary energy, fats are the most concentrated and consist mainly of *empty calories.* Adding fats to already balanced feeds may result in multiple deficiencies of essential nutrients. Optimally, feeds should be fortified with fat at a level of 12 to 19% (120 to 190 g/kg) (Kronfeld et al., 1994). Potential negative associative effects are sometimes a result of added dietary fat. Consequently, it is safest to add fats into feeds and balance the formula accordingly rather than supplementing fat sources to already complete and balanced feeds.

Palatability of Fat

The acceptance of fats in equine diets has been examined in several studies. Holland et al. (1998a) found corn oil to be the most highly palatable form of supplementary fat to the equine diet. The poorest acceptance was received by tallow, which is not surprising considering that the horse diet, throughout evolution, has been negligible in animal fats. Another study that fed animal tallow found that it was palatable by horses (Potter et al. 1992b). However, the tallow that was used in this study did not include sources that were down, disabled, diseased and dead. The renderer was given four days notice and tallow was obtained with special effort. Rice bran has also shown promise as a fat source in equine diets. Horses readily consume this cereal by-product, as it is also highly palatable.

A problem with fat fortified feeds is that it may spoil after a month or two. Efforts to prevent oxidation and rancidity are currently under examination by feed manufacturers (Kronfeld et al. 2001).

Digestibility of Fat

The digestibility by horses of the ether extract (EE) component in feeds varies greatly from forages to grains and further to added fats. This variation is attributed to the presence of non-hydrolysable substances in the EE, especially in leafy foods, and the dilution of endogenous fecal fat. EE is nearly 100% triglyceride in extracted vegetable oils and refined animal fats. The determination of digestibility and associative effects of fats are necessary for accurately determining the digestible energy (DE) of feeds.

Mean estimates of apparent digestibilities (D_A) of EE in feeds consumed by horses and ponies are 42 to 49% for forages, 55 to 76% for grains and 88 to 94% for added fats and oils, and up to 95% for corn oil (Bowman et al., 1979; Kane et al., 1979; Hintz and Schryver, 1989; Sturgeon et al., 2000). The low D_A of EE in forages is usually attributed to the presence of waxes, sterols, pigments and other indigestible lipids (VanSoest, 1982).

Associative Effects of Feeding Fat

Studies have found some negative associative effects associated with high fat diets. Associative effects have been reported with calcium (Rich, 1980), magnesium (Pagan, 1998), fiber (Jansen et al. 2002) and protein (Holland, 1998a). Soybean oil (158g/kg DM) depressed crude fiber digestibility from 71 to 57%, which supports the findings of several previous studies in the same laboratory (Jansen et al., 2002). In another study, the combination of added soybean oil (50g/kg) and soy lecithin (50g/kg) had no effect on crude fiber digestibility (Kronfeld et al., 2001).

Most studies have not shown negative associative effects of fat on protein digestibility (Kronfeld et al., 2001). Adding fats helps achieve desired low protein content in feeds for equine athletes (Graham-Theirs et al., 2001).

Avoiding Equine Grain Related Disorders

Traditionally, the equine diet consists of forage and grain. Physiological studies suggest that concentrates high in sugar and starch ("sweet feeds") for horses should be 2.2 kg or less in order to prevent rapid and abnormal fermentation in the cecum (Potter et al., 1992a; Meyer et al., 1995). However, when there is a demand to increase the percentage of grain and molasses in the diet in order to meet energy requirements, possible digestive complications may occur.

The lipolytic capacity of the small intestine in horses adapts in a few days to several weeks, to adjust to increases in fat intake (Kronfeld et al., 2001). In contrast, sugar and starch hydrolysis is non-adaptive to higher intakes (Potter et al., 1992a; Meyer et al., 1995). The rapid fermentation of concentrates high in sugar and starch lead to excessive production of gas and lactic acid, which is poorly absorbed and accumulates. Colic may result when the accumulating gas and fluid cause the closing of the cecal-colic valve and the distension of the cecum. Lactic acid also lowers the pH leading to acidic colitis and enabling entry of bacteria into the intestinal wall and blood. Acid lysis of bacteria releases endotoxins, which contribute to some forms of laminitis. When sugar and starch are replaced with fat these problems are avoided (Kronfeld et al., 2001).

Exaggerated responses of blood plasma glucose and insulin have been shown in horses fed 2.2 kg meals of "sweet feed" compared to fat-fortified, low starch feeds. Gastric ulcers, extertional-rhabdomyolysis and developmental orthopedic are all associated with this response to high sugar and starch diets in horses. Fat fortified diets may reduce the risk of these disorders in horses (Kronfeld et al., 2001).

Fat Enhanced Performance

Feeding additional fat to performance horses has several beneficial effects. Feeding fat-fortified diets lowers bowel ballast and has additional improved metabolic and regulatory effects (Kronfeld et al., 1994). Diets high in fat also have calming effects on horses, which may improve performance through increased tractability. Reactivity, as responses to pressure, loud noise and sudden visual stimuli, was lower in horses fed high fat diets than in the control diet (Holland et al., 1996a). Studies with growing horses fed high fat diets report improved growth patterns (Scott et al., 1989).

Field trials on endurance horses have shown that horses fed high fat diets had sustained blood glucose concentrations and reduced dehydration (Hintz and Schryver, 1989). Horses trained aerobically on a treadmill and fed 10% added fat demonstrated muscle glycogen sparing. In addition, blood lactate levels were lower after the same aerobic exercise test, thus reducing fatigue (Greiwe et al., 1989).

In high ambient heat and humidity, athletic performance may be affected over middle and long distances. Energy partition studies have shown that heat production was 52% for horses fed high fat diets, compared with 59% in the control group (Kronfeld, 1996).

Substituting fat for soluble carbohydrates reduces heat of fermentation, hence the thermal load (Kronfeld et al., 1998). Fat adaptation also improves the efficiency of the utilization of metabolizable energy (Kronfeld et al., 1994).

Conclusion

The advantages of fat-fortified horse feeds have been strongly supported by several studies throughout the last 30 years, and especially through the last decade. However, feed manufacturers have been conservative in their marketing strategies for equine feeds high in fat. Their reluctance has focused on palatability, digestibility, associative effects, and price.

Objectives

The primary objectives of this study were (1) To determine mean digestibility, fecal output and daily intake of two feeds using alkane markers in 8 horses; and (2) To determine predictability of fecal output, digestibility and daily intake for an individual horse fed two feeds using alkanes markers. A secondary objective of the study was (3) to determine the partial digestibility of ether extract (EE) and other nutrients in the high fat cereal by-product (CBP).

Materials and Methods

Alkanes

Odd-chain alkanes and even-chain alkanes were used as internal and external markers respectively to determine the dry matter digestibility (DMD), fecal output (FO), and dry matter intake (DMI) of two different feeds. Alkane marker methods were compared to total collection measurements. Horses were dosed with 0.5 g of the even-chain alkanes C32 (dotriacontane) and C36 (hexatriacontane) two times per day. The experimental design was a replicated 2 x 2 Latin square using eight healthy mature Thoroughbred geldings at maintenance (597.50 ± 19.52 kg) and two periods of 3 wk. The horses were housed in 4 m^2 stalls for approximately 18 h a day. They were given about 6 h a day of turnout for free-choice exercise in a dry-lot devoid of any vegetation, with unlimited access to water and salt.

Dietary treatments

Eight horses were randomly assigned to two groups consisting of four horses each. Four horses were fed a diet of hay only (H) (Appendix table 1). The hay was a mixture of orchgardgrass (*Dactylis glomerata*) and tall fescue (*Festuca arundinicae*), second cutting baled at the Middleburg Agriculture and Research Extension Center. The nutrient content of the H is listed in Appendix table 6. The remaining four horses were fed the same hay plus a cereal by-product (H+CBP) (Appendix table 1). The cereal by-product was obtained from a commercial food manufacturer. The nutrient composition of the

CBP is listed in Appendix table 5. The feeds were switched for the second period so that the horses that received H in period 1 received H+CBP in Period 2.

Experimental Procedures

A 42 day trial was conducted in September and October 2000 and the experimental design was a replicated 2 x 2 Latin square. Each period was 21 days in length. On d 1, the horses were weighed and placed in $4m^2$ box stalls. Through d 7 horses were accommodated to their respective feeds. On d 8, the horses were weighed again and baseline fecal samples were taken. Dosing of C32 and C36 began on d 11 and ended on d 18. Total collection of feces and feed offered was performed on the last four days of dosing (15 d to 18 d). The last three days of the study were designated for a recovery period and the horses were fed hay only before a crossover of dietary treatments for the second period (19 d to 21 d).

Horses were initially fed to meet their daily energy requirements (NRC, 1989), and hay was adjusted weekly to maintain body weight at a body condition score of approximately 5. Horses were fed their respective feeds twice a day at 700 h and 1500 h. Horses offered H + CBP received 1.3 kg of the cereal by-product (CBP) two times per day at morning and evening feedings. Hay was weighed and fed in a net. Orts from previous feedings were collected and weighed for total collection measurements. All horses were fed 1/3 of their expected daily hay intake at 700 h and the remaining 2/3 at 1500 h.

Samples of hay were taken with a core sampler and grab samples of cereal by-product were collected once a week during the entire study. Duplicate feed samples were sent to Dairy One in Ithaca, New York for nutrient analysis.

Dosing and Sampling.

Horses were dosed twice daily at 700 h and 1500 h for 8 days. Horses offered H + CBP were dosed via the CBP. The even-chain alkanes were dissolved in petroleum spirits and 5.0 g (10.0 ml solution) was thoroughly mixed with 300 g of the 1.3 kg CBP. The specially prepared 300 g CBP plus alkane mixture was set out for the petroleum spirits to evaporate. After the solvent had evaporated it was mixed thoroughly with the remaining 1.0 kg of CBP. Horses offered H were dosed via "granola" bars containing 0.5 g of both C32 and C36. Bars were prepared using small amounts of grain, flour and beer, all with negligible alkane content so as to not alter recoveries. Dosing methods were different between diets because even-chain alkanes could not be mixed in with the hay as the alkane mixture did not adequately adhere to the hay.

Fecal grab samples were collected twice daily during the dosing phase. Total fecal output for 24 h was collected using Equisan nappies (Equisan Ltd, Australia) for 4 d during the last week of each trial. Nappies are fecal and urinary collection bags that attach to the horse with out impeding freedom of movement (Figure 19). Baseline fecal grab samples were collected for 3 d prior to dosing external markers and recovery fecal samples were collected for 4 d after dosing.

Chemical Analysis of Alkanes

Composite feed and fecal samples were dried in a forced air oven and ground through a 1mm screen Wiley Mill (Thomas Wiley model 4, Swedesboro, NJ). The feed samples were collected once per week for the duration of the experiment. Fecal samples were collected on the last 4 d of the dosing period. All samples were composited, mixed thoroughly, and two sub samples were taken for duplicate alkane analysis. The alkane content of the samples was determined by gas chromatography (Model 5890A Hewlett Packard, Wilmington, DE). The methods of analysis were performed according to the methods of Mayes et al. (1984) and Ordakowski (2001) with some minor modifications as follows.

A 0.10 g sample of feed or a 0.30 g sample of feces was placed in a 20 ml Pyrex tube fitted with a screw top prior to the addition of 0.1008 mg of C34 (tetratriacontane) as the internal (chemical) standard (Sigma Chemical, St. Louis, MO). Seven ml of 10% ethanolic KOH (Sigma Chemical, St. Louis, MO) were added to the Pyrex tube. Contents were mixed thoroughly and placed in a 90°C water bath for 3 hours. During this time, the tube contents were mixed thoroughly every 30 min. After the samples were cooled, 7 ml of distilled water and 7 ml of heptane (Sigma Chemical, St. Louis, MO) were added, and again, the tube contents were mixed thoroughly. The organic extract was removed and applied to a silica gel column (Sigma Chemical, St. Louis, MO) that was contained in disposable Oxford pipet tips (200 ul) with glass wool stoppers. The effluent was collected in 20 ml scintillation vials. The column was rinsed with approximately 10 ml of heptane. The samples were placed in a fume hood overnight to allow the eluent to evaporate. The following day, the dried sample was re-dissolved with

1.0 ml heptane before injection of 0.5 ul onto a 30 m x .52 mm x 1.5 ul fused silica capillary column (Supelco Inc. Bellefonte, PA) in the gas chromatograph fitted with a flame ionization detector. The chromatograph carrier gas was helium and it had a flow rate of approximately 9 to 9.25 ml/min. The column oven temperature was programmed at 240°C for 4 min; an increase of 3°C/min to 288°C; 2°C/min to 298°. The area under the peak for each alkane was determined using an integrator (Model 3393A, Hewlett Packard, Wilmington, DE). The identities of the odd-chain alkanes (C25 to C33) and the even-chain alkanes (C32 and C36) were determined from their retention times relative to the internal standard (C34) and with authentic standards. Peak areas were converted to the amounts of alkane by reference to the internal standard.

Total Collection and Alkane Calculations

Total collection DMD (DMD_{TC}, %) of both the H and H + CBP diets was calculated using mean diet intake (DMI, kg/d, DM) and mean fecal output (FO, kg/d, DM) from the last four days of each collection period:

$$DMD_{TC} = (1- (FO/DMI))*100$$

Fecal recovery (R, %) of odd-chain alkanes (C25 to C33) and the dosed even-chain alkanes (C32 and C36) were calculated for each horse and each diet using mean DMI, FO and alkane concentration in the diets (C_I, mg/kg DM) and alkane concentration in the feces (C_F, mg/kg DM):

$$R = ((FO * C_F) / (DMI * C_I)) * 100$$

Estimated DMD (DMD_E) of the diets was calculated using the concentration of the alkane in the feed (C_I) and the concentration of the alkane in the feces (C_F). The alkane chain lengths C29, C31 and C33 were used to estimate total diet digestibility as follows:

$$DMD_E = (1-(C_I / C_F)) * 100$$

Nondigestibility (N) of the diets was estimated as follows:

$$N = 1-DMD_E = C_I / C_F$$

Estimated FO (FO_E) of the diets was calculated using the even-chain alkanes C32 and C36 as external markers. The amount of alkane dosed (Dose, g) and the concentration of the even-chain alkane in the feces ($C_{F(even)}$ g/kg)) were used in the following calculation to estimate daily FO (kg):

$$FO_E = Dose / C_{F(even)}$$

Dry matter intake (DMI_E) for diets was estimated using the double marker method where the mean of C32 and C36 was used as estimates of fecal output with the nondigestibility as estimated by C29, C31 and C33:

$$DMI_E = FO / N \text{ or}$$

$$DMI_E = FO * (C_F / C_I)$$

Statistical Analysis

Data were summarized as means and standard error of the mean (SE). The recovery rates (R), DMD, FO and DMI were analyzed separately using the GLM procedure of SAS (1989) with horse and feed interaction in the model. R, DMD, FO and DMI were then analyzed to compare diet effects with method and feed in the model. Estimates of DMD, FO and DMI as predicted by alkane methods were compared to total collection measurements using the GLM procedure of SAS (1989) as well (with horse and method in the model). Tukey's studentized range test was used to test for differences in alkane recovery, DMD, FO and DMI within and between feeds. Equivalence of TC and alkane estimates of DMD, FO, and DMI were tested by linear regression using graphical software (Slide Write Plus, 1996).

Digestibility

The apparent digestibility (D_A) of nutrients was determined for H and H + CBP diets. In addition, partial digestibility (D_P) of nutrients for the added CBP was determined. Dietary treatments and experimental procedures were the same as for the alkanes.

Chemical Analysis of Feeds and Feces

Composite samples of feeds and feces were submitted in duplicate for wet chemistry analysis of CP, EE, ADF, NDF, NSC (by difference), DE, ash, and Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, and I at the Northeast DHIA Laboratory (Dairy One, Ithaca, NY). The laboratory performed official methods of analysis (AOAC, 1990).

Digestibility Calculations

Partial digestibilities (D_P) were calculated according to Kleiber (1961). D_P of nutrients in the CBP was determined by solving the following equation where the difference in digestible nutrient between the two diets is divided by the difference in total nutrient:

$$D_P = \Delta I - \Delta F/\Delta I$$

Statistical Analysis

Data were summarized as means and standard errors. Data were analyzed by the GLM procedure of SAS (1989) with horse, feed, and period in the model. Tukey's studentized range test was used to test for differences (SAS, 1989).

Results

Alkanes

Recovery of Alkanes

In horses fed H only (Table 1 and Figure 1), recoveries of C32 and C36 were not different ($P = 0.46$) from one another, and the overall mean was 113.0 ± 4.2%. The recoveries of C25 and C27 were different ($P < 0.05$) from those of C29, C31 and C33, which were not different from each other with a combined recovery of 89.2 ± 2.9%. The recovery of C29, C31, C32, C33 and C36 was 98.7 ± 6.2%.

In horses fed H + CBP (Table 2 and Figure 2), no differences ($P < 0.05$) were found between alkanes with different chain lengths. Recoveries were 99.8 ± 3.8, 99.4 ± 13.0 and 99.7 ± 3.9% for odd, even and all chain lengths, respectively. The recovery of all alkanes in both feeds, except C25 and C27 from H, was 99.5 ± 3.2%.

The patterns of recoveries of alkanes of different chain length were different between H and H + CBP feeds (Figure 3).

Alkane Estimates of Digestibility

The DMD was 49.4 ± 1.6 and 52.4 ±1.2 % for the H and H + CBP feeds, respectively, according to TC (Tables 3 and 4, Figures 4 and 5). Corresponding estimates from the C29, C31 and C33 alkanes combined were 40.9 ± 4.3 and 54.7 ± 3.3 % for the H and H + CBP feeds, respectively. These alkane estimates of DMD were 83 and 104% of the TC

estimates for H and H + CBP feeds, respectively. The TC and alkane estimates were not different ($P > 0.05$) from one another (Tables 3 and 4, Figures 4 and 5).

Fecal Output Estimates

The FO was 5.07 ± 0.21 and 5.05 ± 0.26 kg/d of DM for the H and H + CBP feeds, respectively, according to TC (Tables 5 and 6, Figures 6 and 7). Corresponding estimates from C32 and C36 combined were 4.59 ± 0.30 and 4.89 ± 0.56 kg/d. These alkane estimates were 91 and 97% of the TC estimates for H and H +CBP feeds, respectively. The TC and alkane estimates were not different ($P > 0.05$) from one another (Tables 5 and 6, Figures 6 and 7).

Feed Intake Estimates Using the Alkane Double Marker Method

The DMI was 9.72 ± 0.45 and 10.62 ± 0.43 kg/d of DM for the H and H + CBP feeds, respectively, according to TC (Tables 7 and 8, Figures 8 and 9). Corresponding estimates from C32 and C36 combined were 7.84 ± 0.57 and 10.33 ± 0.93 kg/d. These alkane estimates were 81 and 99% of the TC estimates for H and H +CBP feeds, respectively. The TC and alkane estimates were not different ($P > 0.05$) from one another (Tables 7 and 8, Figures 8 and 9).

Equivalence

Alkane data for individual horses were tested for linear regressions on corresponding TC data (Table 9). Regressions were found only for the FO data; the H feed ($P = 0.03$) and the H + CBP feed ($P = 0.06$). The data were compared to lines of identity (Figures 10 to 15); equivalence was evident only for the FO data (Figures 12 and 13), and clearly absent from the DMD and DMI data (Figures 10, 11, 14 and 15).

Calibration of Fecal Output

Total collection fecal output data for individual horses were tested for linear regressions on corresponding alkane data for both feeds individually and the feeds combined. Regressions were found for H ($P = 0.027$), H + CBP ($P = 0.061$) and for the two feeds combined ($P = 0.003$). The data were compared to lines of identity (Figures 16,17 and 18).

Digestibility

Apparent Digestibilities

Apparent digestibilities of nutrients in H and H + CBP are summarized in Table 10. Values for individual horses are presented in the Appendix (Tables 9 and 10). The mean D_A was higher for EE ($P < 0.0001$) and CP ($P < 0.01$), and lower ($P < 0.01$) for NSC ($P < 0.01$) and Ca ($P < 0.0001$), in the H + CBP feed than in the H feed.

Partial Digestibility of CBP

Partial digestibilities of nutrients in CBP are summarized in Table 10. The mean D_P of EE was highest for CBP; H + CBP was higher than H ($P < 0.0001$). The mean D_P of CP and NDF were also highest in CBP; H and H + CBP were not different ($P < 0.0001$). The mean D_P of NSC was lowest for the CBP, while the H and H + CBP feeds were not different ($P < 0.0001$). The D_P of Ca was lowest in the CBP and highest in the H, the feeds and supplement were different from each other ($P < 0.0001$). There were no differences in the mean D_P of ADF, P and Mg for CBP, H and H + CBP ($P = .902$, $P = 0.334$, $P = 0.236$, respectively).

Discussion

Alkanes

The results show that mean recoveries of alkanes were close to 100%, but the range for individual alkanes was wide, and the pattern of recoveries for alkanes of different chain length was inconsistent from feed to feed. The results also indicate that mean estimates of the DMI, DMD and FO of a feed, such as H or H + CBP, are determined with reasonable accuracy by means of alkane markers. In contrast, alkane estimates of DMI and DMD in an individual horse fail to predict corresponding TC estimates. The alkane estimate of FO in an individual horse predicts a TC value with error of 16.4%. The CBP was found to be an excellent source of EE, CP and fiber but a poor source of Ca.

Alkane Recovery

The results show that mean recoveries of alkanes were close to 100%, but the range for individual alkanes was wide, and the pattern of recoveries for alkanes of different chain length was inconsistent from feed to feed. Differences in recovery could have been a result of inconsistent alkane concentrations in the feces due to inadequate mixing in the hindgut of the horse. If the feces from total collection had been mixed thoroughly before grab samples were taken (e.g. in a concrete mixer), the fecal alkane concentrations may have been more consistent. It is also possible that some digestion of alkanes occurred in the hindgut. Studies with ruminants have found that lower chain length alkanes are

digested in the complex reticulo-rumen system (Mayes et al., 1986a; Mayes et al., 1986b).

Similar recoveries were reported for odd-chain alkanes in horses fed tall fescue *(Festuca arundinacae)*/alfalfa *(Medicago sativa)* mixed hay, where rates were 88 to 90% (Ordakowski et al., 2001). Horses were fed orchardgrass *(Dactylis glomerata)*/alfalfa mixed hay and recoveries were 75 to 92% (Ordakowski et al., 2001). In this study, horses were offered mixed grass hay containing orchardgrass and tall fescue, which confirms reasonable recovery rates when compared to previous studies. The pattern of recovery was typical for the forage species that were present in the hay fed (Malossini et al., 1990).

When a supplement was added to the ration, Ordakowski et al. (2001) also found that C31, C29 and C33 were the most abundant alkanes recovered in the feces of horses that were fed similar diets. However, recovery rates in that study were lower when a fat/fiber supplement containing the CBP was added to the orchard grass/alfalfa diet (71 to 81%). The CBP fed in this study, though not a typical concentrate, did not affect the fecal concentrations of alkane recovery.

In ruminants, Mayes et al. (1986a) found the concentrations of other odd-chain alkanes were reduced when concentrates were added to the diet. In this study, fecal concentrations of alkanes were actually higher in the H + CBP diet, although differences were not significant. With the exception of C27, Mayes et al. (1986a) reported that fecal recoveries were unaffected by diet when sheep were offered perennial ryegrass or perennial ryegrass and barley-based concentrate. A difference in recovery between diets was also not expected since horses were offered the same hay, hence, the same alkane

content. For the most part, the concentrations of alkanes in the CBP were very low; therefore fecal recoveries reflect the alkanes present in the hay that was offered.

The results show that the recoveries of C32 and C36 were higher than the odd-chain alkanes and close to 100%. While the fecal recoveries of odd-chain alkanes was expected to vary due to differences in herbage concentrations, the fecal recovery of dosed synthetic even-chain alkanes is not expected to be affected by diet (Lewis et al., 2002; Dove and Mayes, 1991). Other studies with horses have found higher recoveries of odd-chain alkanes than even-chain alkanes (O'Keefe and McMeniman, 199). The majority of studies have examined ruminants, it is possible that their recoveries are lower due to some alkane digestion in the complex reticulo-rumen system. The recoveries of both C32 and C36 in this study are higher here than reported in other studies with ruminants (Mayes et al., 1986a; Unal and Garnswothy, 1999).

The presence of the CBP supplement did not affect the fecal concentrations of dosed alkanes. Mayes et al. (1986a) found similar recoveries to forage diets when they added concentrates to the rations of sheep. In this study, the addition of CBP, though not a typical concentrate, did not affect recovery rate of the even-chain alkanes C32 and C36.

Alkane Estimates of Digestibility

The results indicate that mean estimates of DMD of a feed, such as H or H + CBP, are determined with reasonable accuracy by means of alkane markers. In contrast, alkane estimates of DMD in an individual horse fail to predict corresponding TC estimates. This can be attributed to variations in hindgut mixing or digestion of alkanes. Similar results

have been reported in previous studies estimating DMD of feeds in horses and other animals (Dove and Coombe, 1992; Ordakowski et al., 2001; Hatt et al., 2001).

Ordakowski et al. (2001) did not find any significant differences between alkane estimated DMD and DMD_{TC} when horses were offered hay. DMD estimated by alkanes underestimated DMD_{TC} in the study by Ordakowski et al. (2001) when horses were offered a diet containing a supplement, which was attributed to incomplete fecal recoveries of alkane internal markers. In this study, fecal recovery rates of C29, C31 and C33 were higher in the H + CBP diet than in the H diet, therefore, it is to be expected that the DMD was more accurate when compared to DMD_{TC}. The high digestibility of H + CBP compared to H is expected as the CBP is like a concentrate in that it is less fibrous than hay and easier to break down in the gastrointestinal tract.

To use plant alkanes as digestibility markers to obtain accurate estimates of DMD, it may be necessary to adjust initial fecal alkane concentrations using the mean recovery of each odd-chain alkane marker (Ordakowski et al., 2001). Estimates of DMD using internal markers are based on the principle that 100% of the fecal marker is recovered. Since the fecal recoveries varied just below 100% for the hay diet and just above 100% for the hay + CBP diet, DMD estimates in this study may have been more accurate for horses fed hay only had the recoveries been adjusted as in other studies (Dove and Coombe, 1992; Ordakowski et al. 2001).

Fecal Output Estimates

The results indicate that mean estimates of FO of horses fed either H or H + CBP are determined with accuracy by means of alkane markers. The alkane estimate of FO in an

individual horse predicts a TC value with error of 16.4%. When both feeds were accounted for in the calibration, alkane predictions of FO were closer to total collection values because the sample size was two-fold compared to the H and H + CBP alone. From the results we can infer that either even-chain alkane, C32 or C36, would be sufficient in estimating FO to be used in double marker methods with internal odd-chain alkanes to estimate the intake of feed and forage in the horse. Similar results have been reported in other studies (Mayes et al., 1995).

It can then be assumed that FO estimates were not affected by the method of dosing the even-chain alkane external marker. These results are justified by a study where even-chain alkanes were dosed by two different methods. One method directly placed the alkanes in the rumen of sheep; the other method dosed the alkanes in suspension form (in xanthum gum) via a dosing syringe. No concentration differences were detected in the feces (Marais et al. 1996).

Alkane Intake Estimates Using the Double Marker Method

The results indicate that mean estimates of DMI of a feed, such as H or H + CBP, are determined with reasonable accuracy by means of alkane markers. In contrast, alkane estimates of DMI in an individual horse fail to predict corresponding TC estimates. This can be attributed to variations in hindgut mixing or digestion of alkanes. Previous studies have shown that C36 is less satisfactory than C32, but it is still useful in estimating FO since it has a relatively high recovery rate of approximately 95% in ruminants (Mayes et al., 1986b). In this study the mean FO estimate from both C32 and C36 was used in the intake calculation. In other studies, a single even-chain alkane was used (Vulich et al.,

1991; Duncan et al., 1999; O'Keefe and McMeniman, 1998; Hatt et al., 2001). Results of experiments have suggested that similar estimates of DMI can be obtained by using C32 or C36 as the external marker, and C33 as the internal marker (Mayes et al., 1986a; Stakelum and Dillon, 1990; Nash, 2001).

Intake of pasture by weanling thoroughbreds was determined with limited success as overestimations of DMI were a result of seasonal differences in pastures. When intakes were compared in captive giraffes using alkanes in the double marker method, intake of a hay diet was underestimated, however, intakes of supplements were similar to total collection measurements (Hatt et al., 1998).

It is possible that DMI as measured by TC procedures overestimated intake because of inaccurate collection of orts. Horses were fed hay via hay bags, and some horses had hay loss to the stall floor, which was collected as orts. However, the underestimation of hay intake applying the alkane double marker method is concurrent with other studies (Hatt et al., 1998). In their study with captive giraffes, Hatt et al. (1998) concluded that the alkanes predicted the intakes of cabbage and cattle pellets accurately, but their estimates of oat/wheat mix and clover hay were significantly lower than direct measurements of hay offered to the animals. Lewis et al. (2002) found that the alkane double marker method accurately predicted intake of ryegrass hay, but slightly overestimated intake when Lucerne hay was fed. Perhaps variation in forage species affects the reliability of the method.

These results are supported by a study in which sheep were fed perennial ryegrass or perennial ryegrass plus concentrates. Sheep were dosed with C32 and intake was accurately estimated in combination with C33 (Mayes et al., 1986a). Hameleers and

Mayes (1998) successfully used C36 in combination with odd chain internal alkanes to predict DMI of supplementary grass silage in grazing dairy cows.

Some studies have suggested that in order for the dual alkane marker technique to be reliable, estimates of the recovery rates need to be more accurate (Lewis et al., 2002). Diurnal concentrations of the dosed even-chain alkanes have been reported, which could potentially result in inaccurate estimates of intake (Dillon and Stakelum, 1990; Stakelum and Dillon, 1990; Dove and Mayes, 1991). Dosing external alkanes once per day, rather than twice per day, increases the variation with time in the concentration of the dosed alkane (Dove and Mayes, 1991). Since the horses in this study were dosed twice per day, variation in alkane concentrations should be limited, although additional doses may enhance accuracy. The downside to dosing more than once per day is that handling the animals more may affect their digestive performance. Since the horses in this study were acclimated to being in stalls and handled routinely, it is unlikely that dosing twice per day affected their feeding and digestive behaviors.

Conclusion

Recoveries of odd and even-chain alkanes in the feces of horses offered both diets were similar. Although none of the alkanes were recovered at exactly 100%, their relative recoveries can still be used to estimate intake in the double marker technique. The wide range of recoveries is most likely due to incomplete mixing in the hindgut and digestion of alkanes in the horse as found in other species (Mayes and Dove, 1991; Ordakowski et al., 2001). This may explain the slight underestimations of digestibility as

predicted by alkanes. In addition, the horses fed H were dosed via a specially prepared cookie that was fed directly to the horse by hand. The horses offered H + CBP were dosed via the CBP, which was placed in a feed pan. It is expected that differences in dosing would impact their recoveries in the feces. In addition, among the 8 horses studied, there was some variation in physiological states in that some were extremely hard keepers, most likely with more inefficient digestive strategies. Overall, the estimates of mean DMD, FO and DMI were in agreement with other studies. It is possible that alkanes could be applied as markers to determine pasture intake in groups of horses, however, intake and digestibility estimates from alkane markers of individual horses may be more difficult to attain.

In addition, this study predicted mean DMD, FO and DMI that were similar to total collection results, however it was not as successful at determining these factors for each individual horse. This poses a problem if the objective is to determine the digestive performance of an individual horse rather than a group of horses.

Digestibility

Partial digestibility of CBP

Fat. The ether extract (EE) value of 85% for CBP compares to 55% for forages and 95% for added triglycerides in 18 previous trials of corn oil, etc. (Kronfeld et al., 2001) The low D_P of EE in forages is usually attributed to the presence of waxes, sterols, pigments and other indigestible lipids (VanSoest, 1984). Forages contain about 58%TG with 95% digestibility and 42% indigestible EE. Similarly, the EE of CBP may contain

about 88% triglyceride and 12% indigestible EE (waxes, pigments, sterols, etc).

Protein. The crude protein (CP) value of 79% for CBP is high in the range for horse feed ingredients (NRC, 1989). Combined with the excellent amino acid profiles, these data can support a claim for high quality protein in CBP.

Fiber. The D_P of neutral detergent fiber (NDF) (59%) is also high in the range for horse feed ingredients (NRC, 1989). The acid detergent fiber (ADF) data were erratic, and 2 negative values (presumably arising from poor analysis) were dropped in calculating the mean and SE (Table 9 and Appendix Table 9).

Soluble Carbohydrates. The nonstructural carbohydrate (NSC) value of 62% for CBP is lower than expected. It probably should represent 100% hydrolysis of sugar and non-resistant starch, plus about 60% fermentation of resistant starch, oligosaccharides and soluble fibers (Kronfeld, 1998). It brings into question, however, the digestibility of starch in PCBP.

Minerals. The ash and Ca, P and Mg values are not remarkable. Erratic results are expected for trace minerals (Fe, Zn, Cu, Mn and I assays often with CVs of 30%) but not sodium and potassium (Appendix table 11).

Associative Effects

Fat. The positive effect on EE is explained by the high digestibility of triglycerides in CBP. CBP is most likely lower in indigestible fat components such as pigments and sterols such as in forage species.

Protein. The positive effect of CBP on crude protein is attributable to the high-quality protein in CBP. It would not be due to the added fat, which tends, if anything, to lower digestibility of CP (Potter et al., 1992; Kronfeld et al., 2001).

Fiber. The lack of a negative associative effect on NDF and ADF confirms that no triglycerides reach the large intestine, where it would interfere with fermentation and fiber digestion. These data reinforce the previous conclusion from 16 trials of added corn oil, etc, that the true digestibility of TG is about 100% in the horse as in other species (following accommodation) (Kronfeld et al., 2001). Other studies have shown that fat depresses the digestibility of fiber in exercising horses (Jansen et al., 2002)

Minerals. The negative associative effect of CBP on ash is explained fully by the effect on calcium. The increase in fecal calcium could be attributable to binding with fatty acids and, perhaps, phytin (Rich, 1980). These data suggest that horse feeds, including CBP, should be fortified with calcium at 1.6-times the NRC recommendations. In the absence of similar data, it would be prudent to apply this overage to other divalent

metals, such as iron, zinc, copper and manganese.

Conclusion

CBP granules are a highly suitable ingredient for all horse feeds, except those designed with a low glycemic index for horses identified at risk of developmental orthopedic disease and the equine rhabdomyolysis syndrome.

CBP should be especially beneficial for exercise, pregnancy and lactation for which a combination of highly digestible fat, high quality protein and a moderate glycemic index has metabolic advantages.

Implications

Alkanes

The use of odd-chain alkanes and dosed even-chain alkanes may accurately predict mean pasture intake in a group of grazing horses as intake estimates in this stall trial have shown promising results when the means are used. However, individual intake estimates may be more difficult to attain. Under grazing situations, dosing methods of the even-chain alkanes should be consistent as one of the methods in this study resulted in overestimation of fecal recovery.

Digestibility

The CBP EE had a mean partial digestibility of 85.4%, indicating that it would be a useful component in feed formulation as a high fat supplement for equines. With a negative associative effect on ash, horse feeds, including CBP, should be fortified with calcium. CBP granules are a highly suitable ingredient for all horse feeds, however, feeds designed with a low glycemic index for horses identified at risk of developmental orthopedic disease and the equine rhabdomyolysis syndrome should avoid CBP.

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Table 1. Fecal Recovery (R,%) of odd and even-chain alkanes for horses fed hay (H).

	H						
	Alkanes						
Horse	C25	C27	C29	C31	C32	C33	C36
1	109.89	92.01	113.24	111.53	124.39	100.40	119.97
2	27.02	60.65	90.25	91.70	99.97	91.67	109.21
3	38.93	62.95	93.68	97.16	121.66	137.68	135.29
4	54.80	62.81	91.35	87.01	105.72	83.46	115.15
5		59.09	83.46	88.43	101.46	39.20	113.15
6	40.92	89.09	124.65	115.43	97.11	47.10	90.28
7	43.24	23.82	69.61	71.82	109.38	80.78	101.40
8	73.61	62.43	73.30	68.61	110.53	88.43	152.47
Mean±SE[a]	55.49 ± 11.5[d]	64.11± 7.40[cd]	92.44 ± 6.61[bc]	91.46 ± 5.90[bc]	108.78 ± 3.51[ab]	83.59 ±10.86[bc]	117.11 ± 6.86[a]

[a,b,c,d,] Means within row with different superscripts differ ($P < 0.05$), (n = 8)

Table 2. Fecal recovery (R,%) of odd and even-chain alkanes for horses fed hay + CBP (H + CBP).

	H + CBP						
	Alkanes						
Horse	C25	C27	C29	C31	C32	C33	C36
1	69.63	54.50	75.55	88.88	84.28	67.17	61.64
2	75.50	83.23	107.44	105.12	138.37	0.00	29.74
3	162.21	142.87	145.97	131.29	120.08	155.14	117.99
4	119.86	107.89	107.60	100.25	95.55	93.77	87.63
5	59.22	65.19	91.41	100.97	120.48	125.78	96.09
6	124.17	72.50	126.25	118.57	95.75	140.99	82.13
7		69.07	97.87	92.64	116.16	82.48	107.32
8	80.03	113.86	118.87	107.67	128.65	129.31	109.11
Mean ± SE	98.66±13.25[a]	88.64±10.64[a]	108.87±7.70[a]	105.67± 4.87[a]	112.41± 6.59[a]	99.33 ±17.76[a]	86.46 ±10.25[a]

[a] Means within row with different superscripts differ ($P < 0.05$), (n = 8)

Table 3. Total collection DMD (DMD_{TC}, %) and estimated DMD (DMD_E,%) from the alkanes C29, C31 and C33 for horses fed hay (H) (kg/d).

	H			
		DMD_E		
Horse	DMD_{TC}	C29	C31	C33
1	44.95	51.39	50.64	45.17
2	44.36	38.35	39.32	39.30
3	47.78	44.26	46.26	62.07
4	54.16	49.82	47.32	45.08
5	48.38	38.15	41.63	31.68
6	45.87	56.58	53.11	14.92
7	55.31	32.54	34.61	41.86
8	54.27	22.47	17.17	35.73
Mean±SE	49.39 ± 1.6[a]	41.69 ± 3.93[a]	41.26 ± 4.05[a]	39.48± 4.74[a]

[a] Means within row with different superscripts differ ($P < 0.05$), (n = 8)

Table 4. Total Collection DMD ($DMD_{TC,}$ %) and estimated DMD (DMD_E,%) from the alkanes C29, C31 and C33 for horses fed hay + CBP (kg/d).

	H + CBP			
		DMD_E		
Horse	DMD_{TC}	C29	C31	C33
1	51.69	36.05	45.64	28.08
2	48.44	52.00	50.95	
3	49.90	65.68	61.84	67.71
4	58.62	61.54	58.72	55.87
5	53.41	49.03	53.86	62.96
6	49.27	59.82	57.22	64.02
7	53.71	52.70	50.03	43.87
8	54.51	57.81	57.07	64.82
Mean±SE	52.44± 1.18[a]	54.33± 3.25[a]	54.42± 1.88[a]	55.33 ± 5.11[a]

[a] Means within row with different superscripts differ ($P < 0.05$), (n = 8)

Table 5. Fecal output from total collection (FO_{TC}) and estimated fecal output from the even-chain alkanes C32 and C36 (FO_E) (kg/d) for horses fed hay (H).

	H		
		FO_E	
Horse	FO_{TC}	C32	C36
1	5.94	4.69	5.09
2	5.23	5.14	4.93
3	4.94	3.99	3.76
4	4.13	3.84	3.69
5	4.94	4.79	4.49
6	5.18	5.24	5.90
7	5.72	5.14	5.80
8	4.45	3.96	3.00
Mean ± SE	5.07 ± 0.21[a]	4.60 ± 0.21[a]	4.58 ± 0.37[a]

[a] Means within row with different superscripts differ ($P < 0.05$), (n = 8)

Table 6. Fecal output from total collection (FO_{TC}) and estimated fecal output from the even-chain alkanes C32 and C36 (FO_E) (kg/d) for horses fed hay + CBP (H+CBP).

	H + CBP		
		FO_E	
Horse	FO_{TC}	C32	C36
1	5.96	7.12	8.85
2	5.52	4.02	
3	5.04	4.23	3.91
4	4.07	4.29	4.25
5	5.19	4.34	4.94
6	5.21	5.48	5.80
7	5.56	4.82	4.74
8	3.88	3.04	3.25
Mean ± SE	5.05 ± 0.26[a]	4.67 ± 0.40[a]	5.11 ± 0.69[a]

[a] Means within row with different superscripts differ ($P < 0.05$), (n = 8)

Table 7. Total collection dry matter intake (DMI_{TC}) and estimated dry matter intake from the alkanes C29, C31 and C33 (DMI_E) for horses fed hay (H).

	H	
Horse	DMI_{TC}	DMD_E
1	9.40	9.64
2	9.46	8.25
3	9.57	8.12
4	9.57	7.17
5	10.79	6.32
6	9.00	9.86
7	12.18	8.63
8	7.83	4.70
Mean ±SE	9.72 ± 0.45^a	7.84 ± 0.57^a

[a,] Means within row with different superscripts differ ($P < 0.05$), (n = 8)

Table 8. Total collection dry matter intake (DMI_{TC}) and estimated dry matter intake from the alkanes C29, C31 andC33 (DMI_E) for horses fed hay + CBP.

	H + CBP	
Horse	DMI_{TC}	DMI_E
1	12.34	15.24
2	9.84	8.05
3	12.01	10.21
4	8.53	9.24
5	10.71	10.36
6	10.07	13.05
7	11.14	9.23
8	10.27	7.28
Mean ±SE	10.62 ± 0.43^a	10.33 ± 0.93

[a] Means within row with different superscripts differ ($P < 0.05$), (n = 8)

Table 9. Linear regressions for equivalence of alkane and total collection data for individual horses.

	y = a +bx			
Diet	y	Regression Equation	r	*P* value
H	DMD	80.32 – 0.800x	0.430	0.29
H + CBP	DMD	47.20 + 0.140x	0.525	0.90
H	FO	0.574 + 1.019x	0.766	0.027
H + CBP	FO	-2.18 + 1.380x	0.461	0.060
H	DMI	4.308 + 0.363x	0.270	0.520
H + CBP	DMI	1.051 – 1.071x	0.501	0.210

Table 10. Apparent digestibility of nutrients in H (H_{DA}) and H + CBP ($H + CBP_{DA}$); Partial digestibility of nutrients in CBP (CBP_{DP}) [ab].

			H_A		$H+CBP_A$		CBP_P	
Nutrient	*t*	*P*	Mean	SE	Mean	SE	Mean	SE[b]
EE	10.3	**0.000**	30.0	3.1	71.0	2.0	85.4	26
CP	3.5	**0.010**	67.5	1.2	70.8	1.0	79.3	2.6
ADF	0.58	0.58	32.5	1.9	33.4	1.3	38.0	15.5
NDF	1.98	0.088	40.1	1.6	42.7	1.2	59.7	8.5
NSC	3.51	**0.010**	95.6	3.2	85.5	2.4	62.2	7.6
Ash	6.07	**0.000**	48.4	2.2	41.2	1.6	26.6	2.6
Ca	6.45	**0.000**	51.3	1.8	40.4	1.1	30.4	2.1
P	1.76	0.12	9.2	2.2	11.8	1.6	13.4	2.0
Mg	1.59	0.16	31.3	2.0	28.8	1.3	26.9	1.8

[a]Analysis (AOAC, 1990) performed by Dairy One, Ithaca, NY; [b](n = 8)

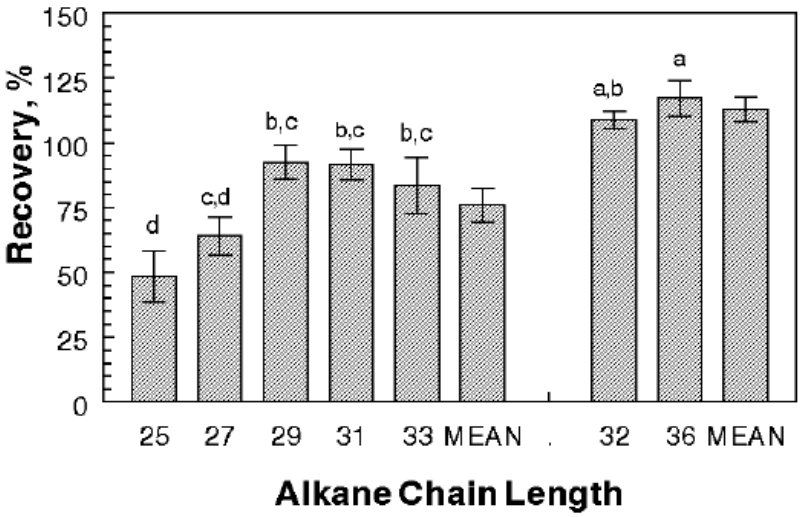

Figure 1. Fecal recovery (R,%) of odd and even-chain alkanes for horses fed hay (H).

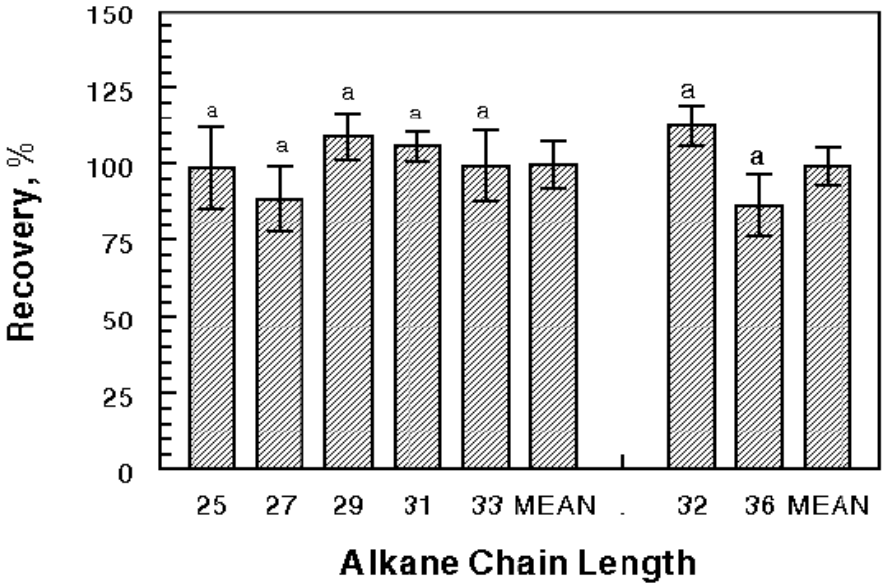

Figure 2. Fecal recovery (R,%) of odd and even-chain alkanes for horses fed hay and CBP (H + CBP).

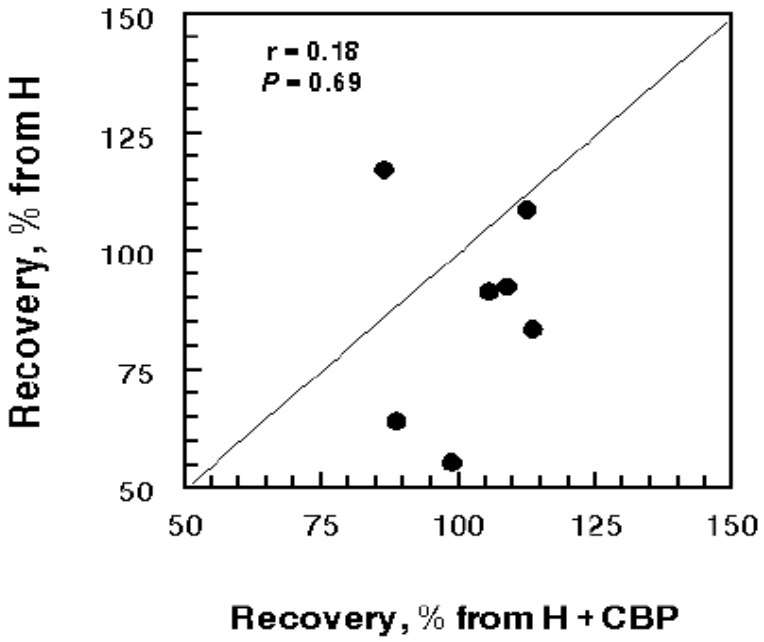

Fig 3. Patterns of recoveries (R, %) of alkanes were inconsistent between hay (H) and hay and CBP (H + CBP).

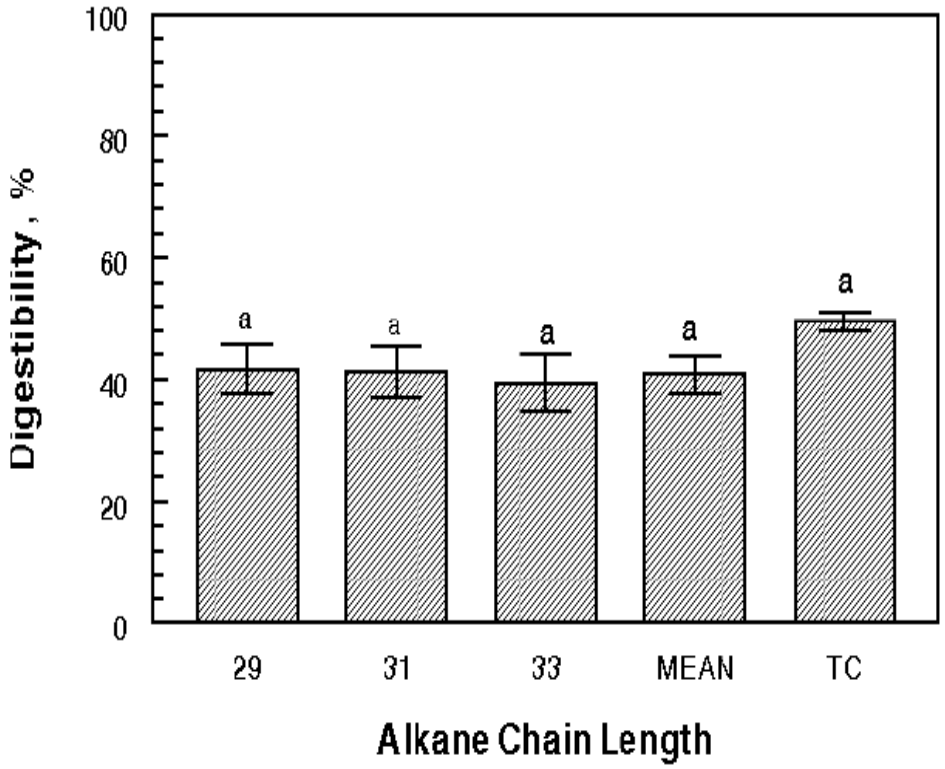

Figure 4. DMD_E from odd-chain alkanes C29, C31 and C33 compared to DMD_{TC} for the hay (H).

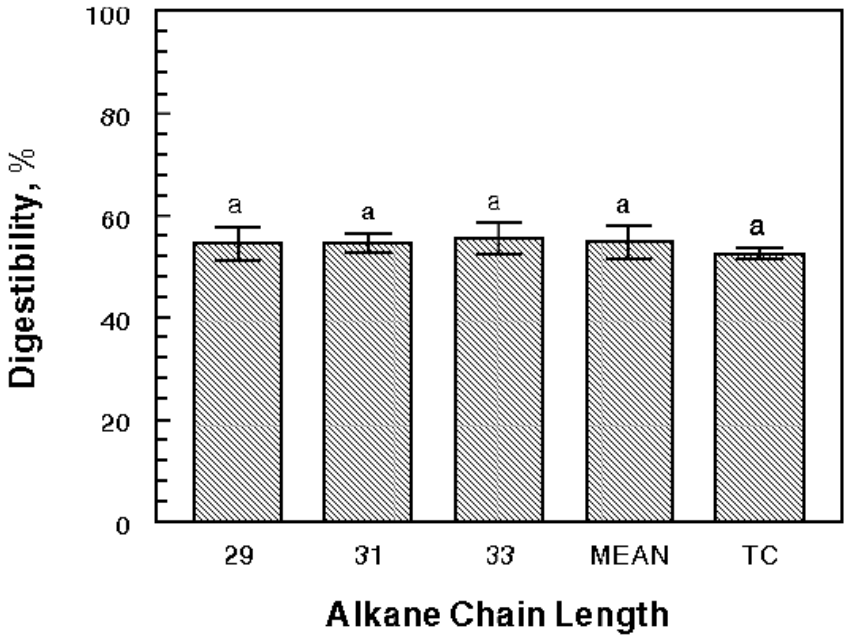

Figure 5. DMD_E from odd-chain alkanes C29, C31 and C33 compared to DMD_{TC} for the hay and CBP (H + CBP).

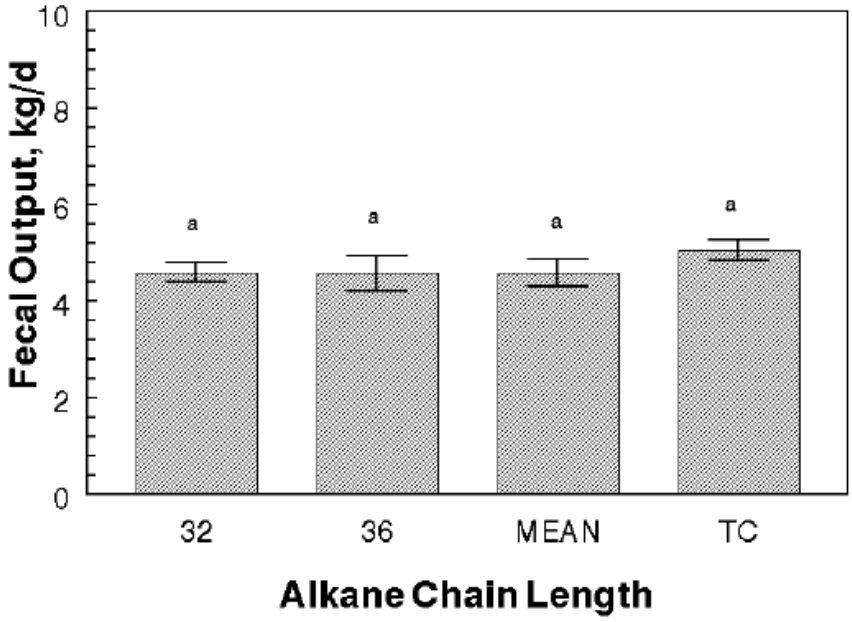

Figure 6. FO_E from even-chain alkanes C32 and C36 compared to FO_{TC} for the hay (H).

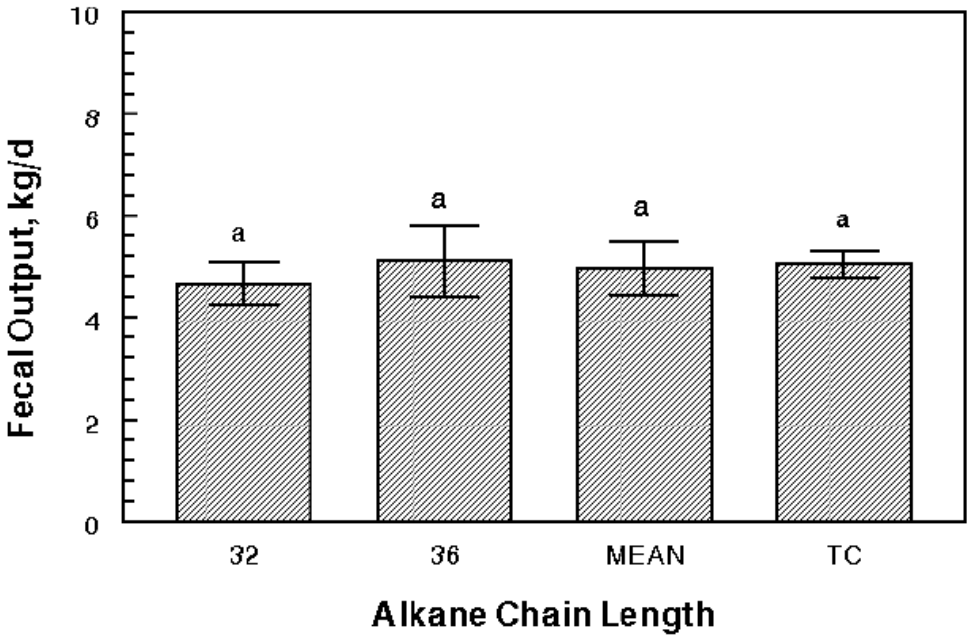

Figure 7. FO_E from even-chain alkanes C32 and C36 compared to FO_{TC} for the hay and CBP (H + CBP).

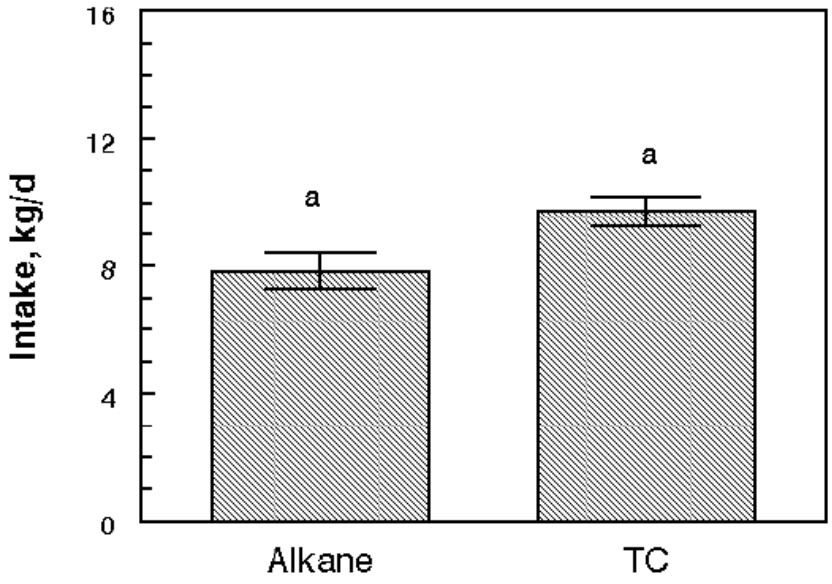

Figure 8. DMI_E (kg/d) for alkanes compared to DMI_{TC} for the hay (H).

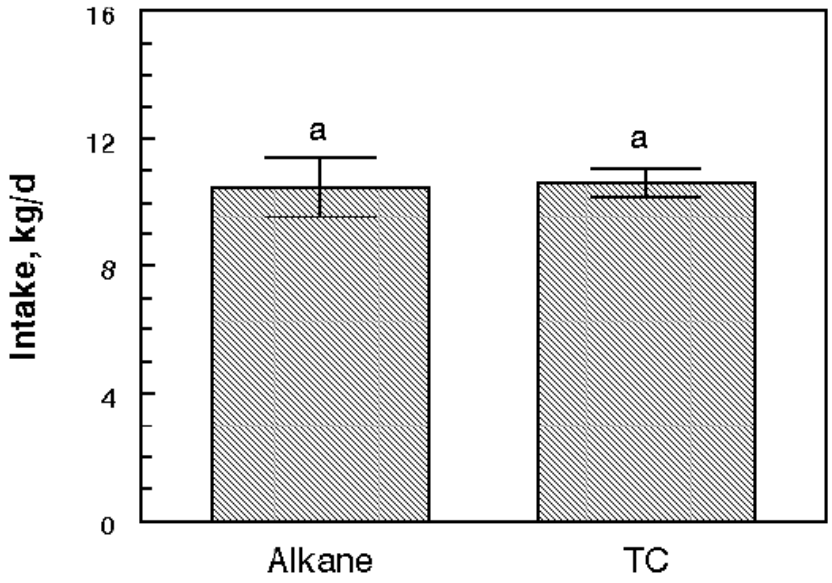

Figure 9. DMI_E (kg/d) for alkanes compared to DMI_{TC} for the hay and CBP (H + CBP).

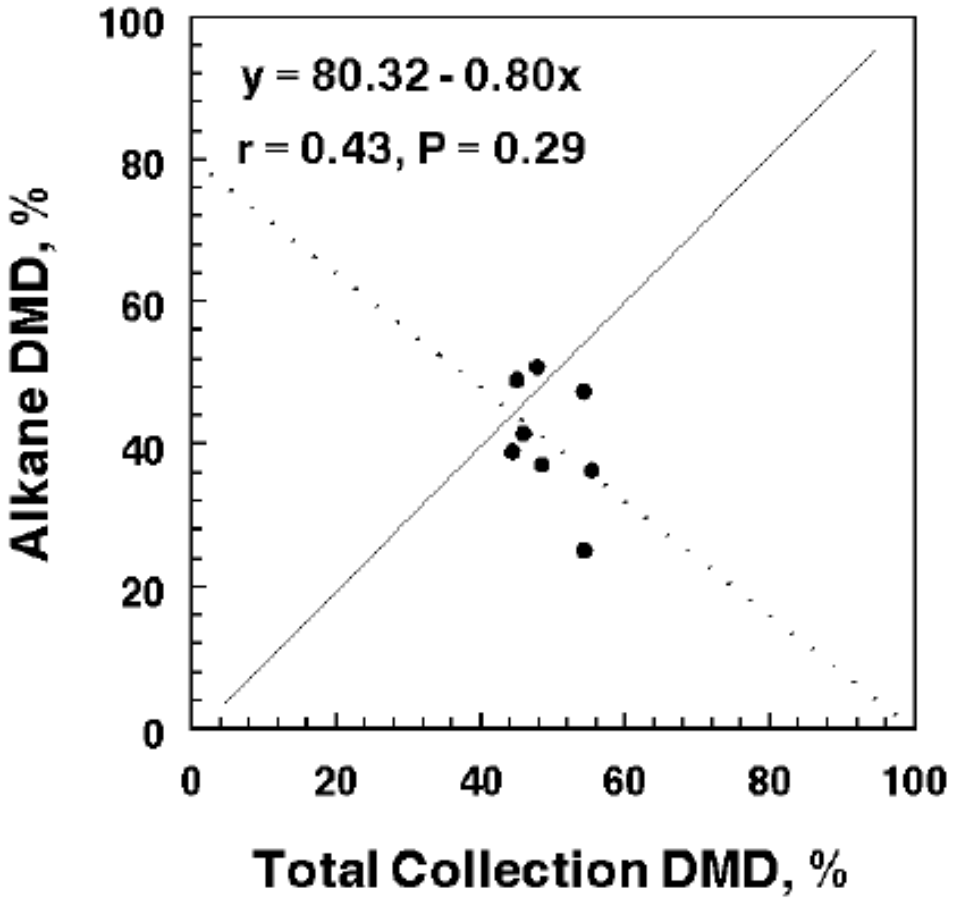

Figure 10. Non-equivalence relationship between DMD_E and DMD_{TC} for the hay (H). Solid circles represent individual horses.

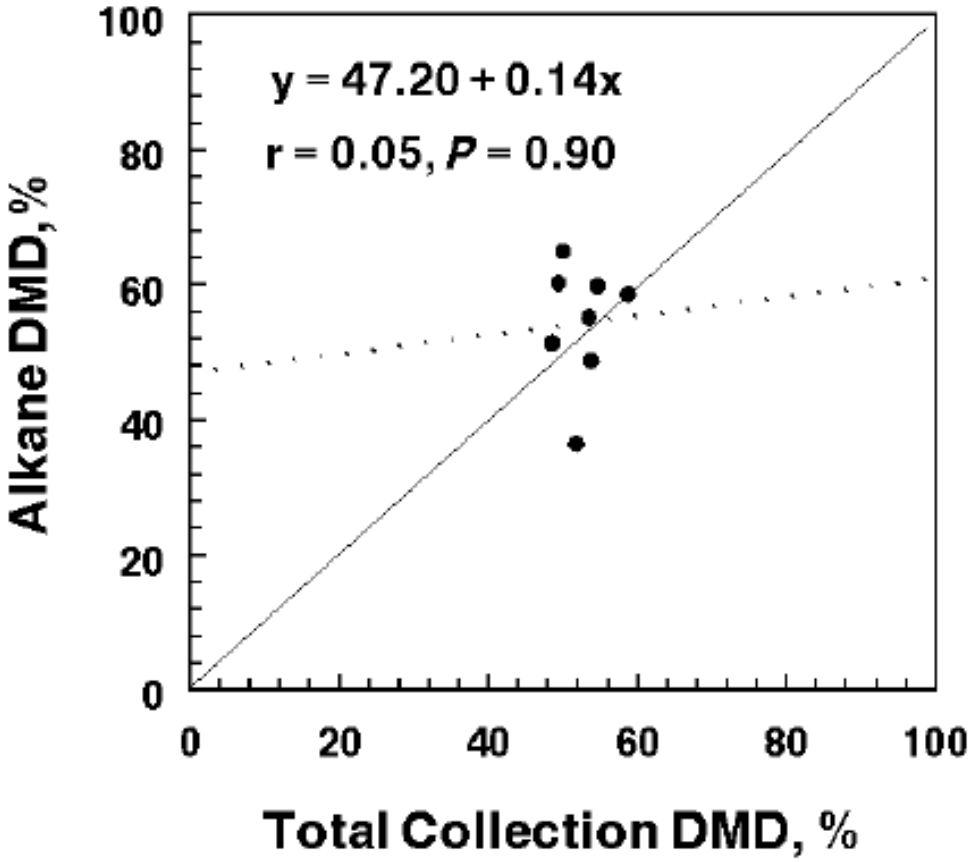

Figure 11. Non-equivalence relationship between DMD_E and DMD_{TC} for the hay and CBP (H + CBP). Solid circles represent individual horses.

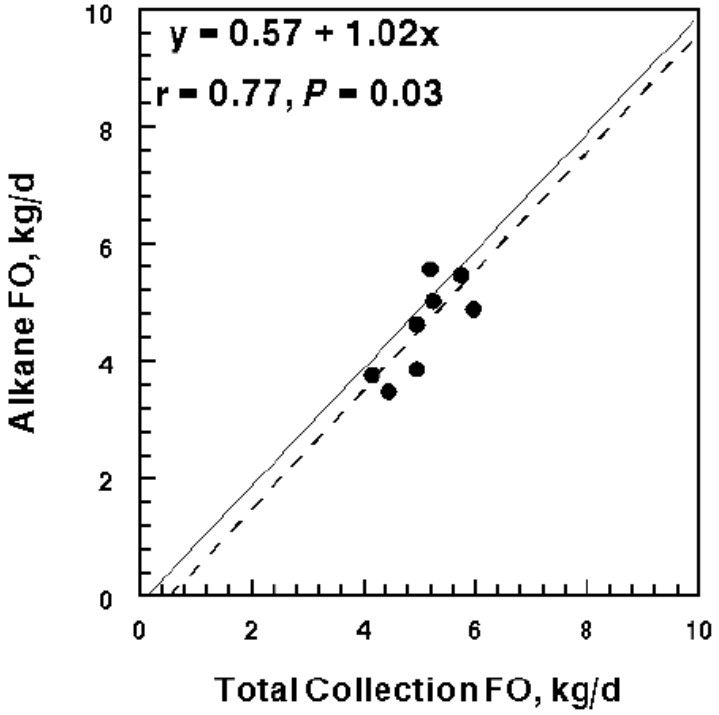

Figure 12. Equivalence of FO_E and FO_{TC} for the hay (H). Solid circles represent individual horses.

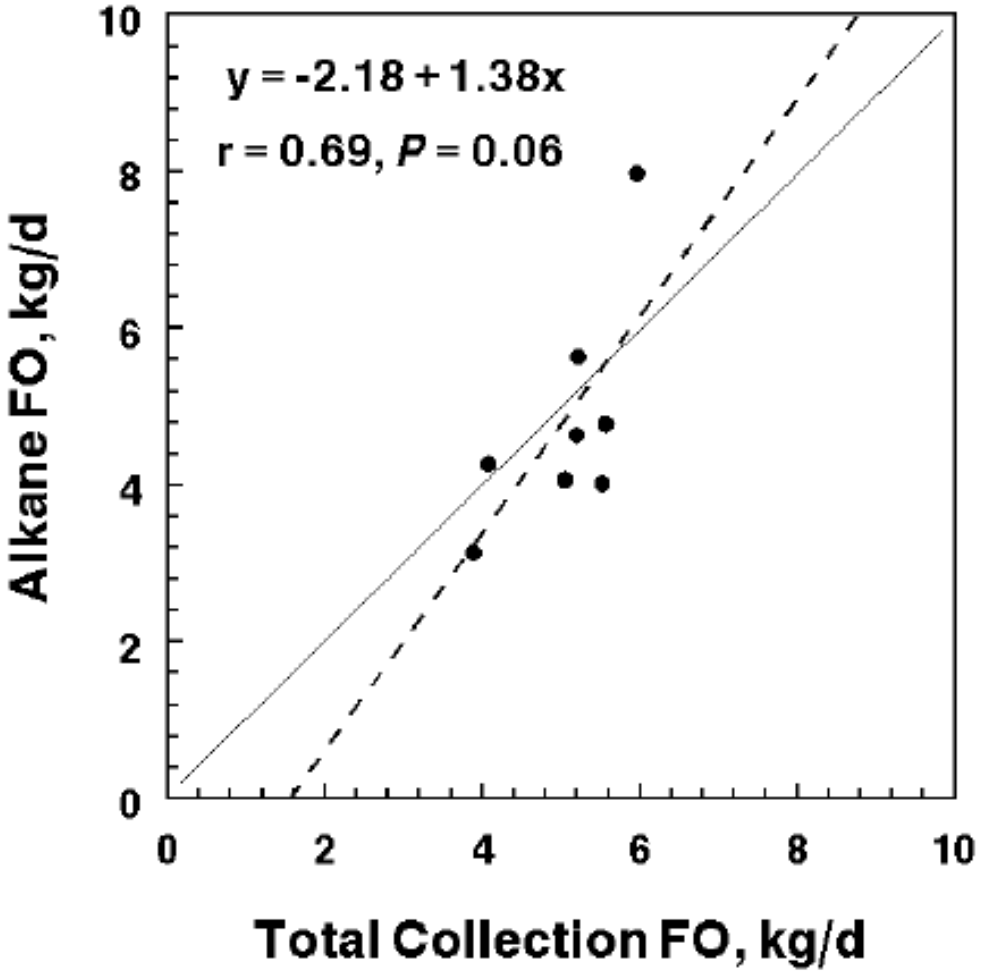

Figure 13. Non-equivalence relationship between FO_E and FO_{TC} for the hay and CBP (H + CBP). Solid circles represent individual horses.

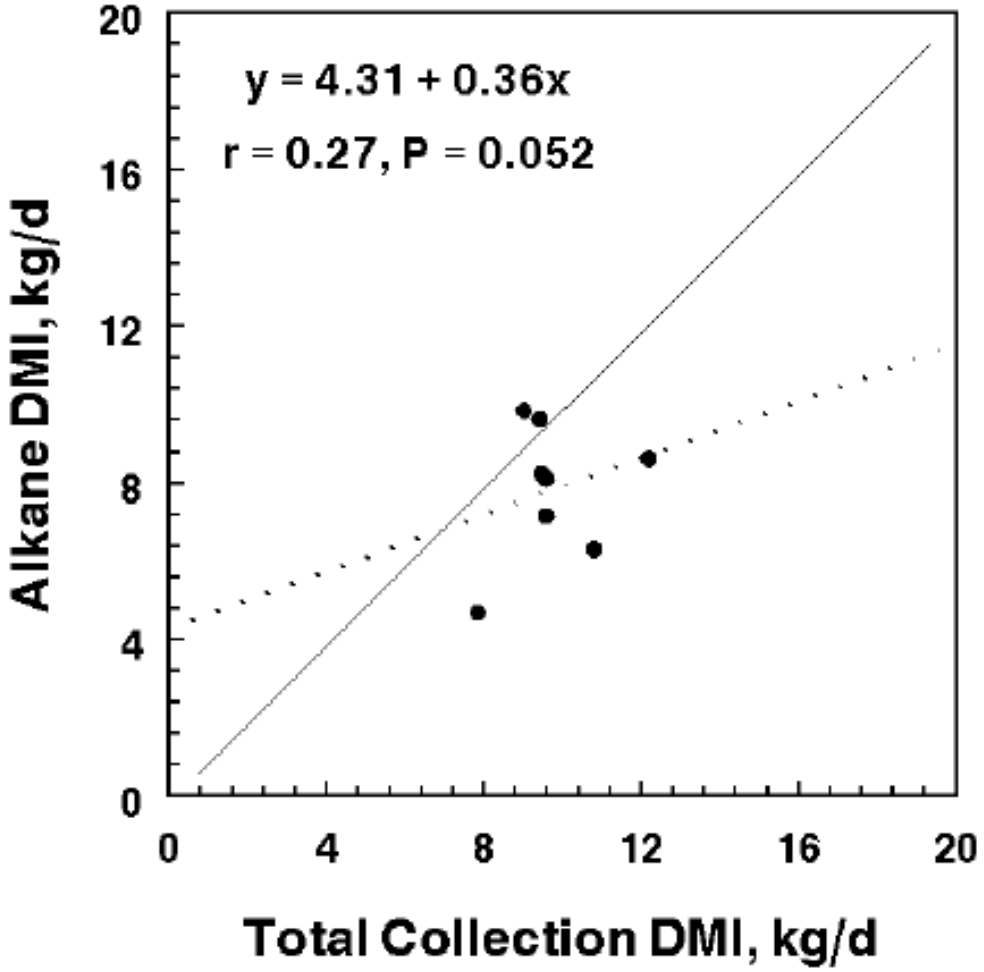

Figure 14. Non-equivalence relationship between DMI_E and DMI_{TC} for the hay (H). Solid circles represent individual horses.

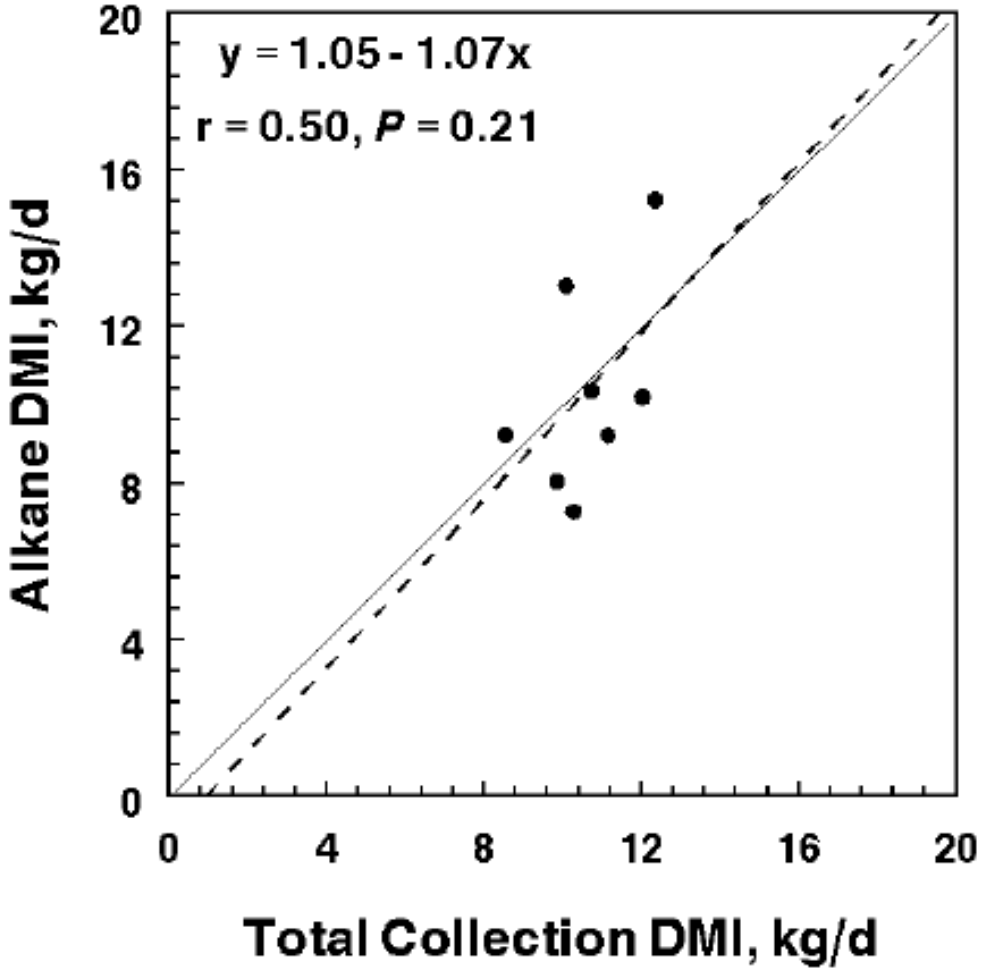

Figure 15. Non-equivalence relationship between DMI_E and DMI_{TC} for the hay and CBP (H + CBP). Solid circles represent individual horses.

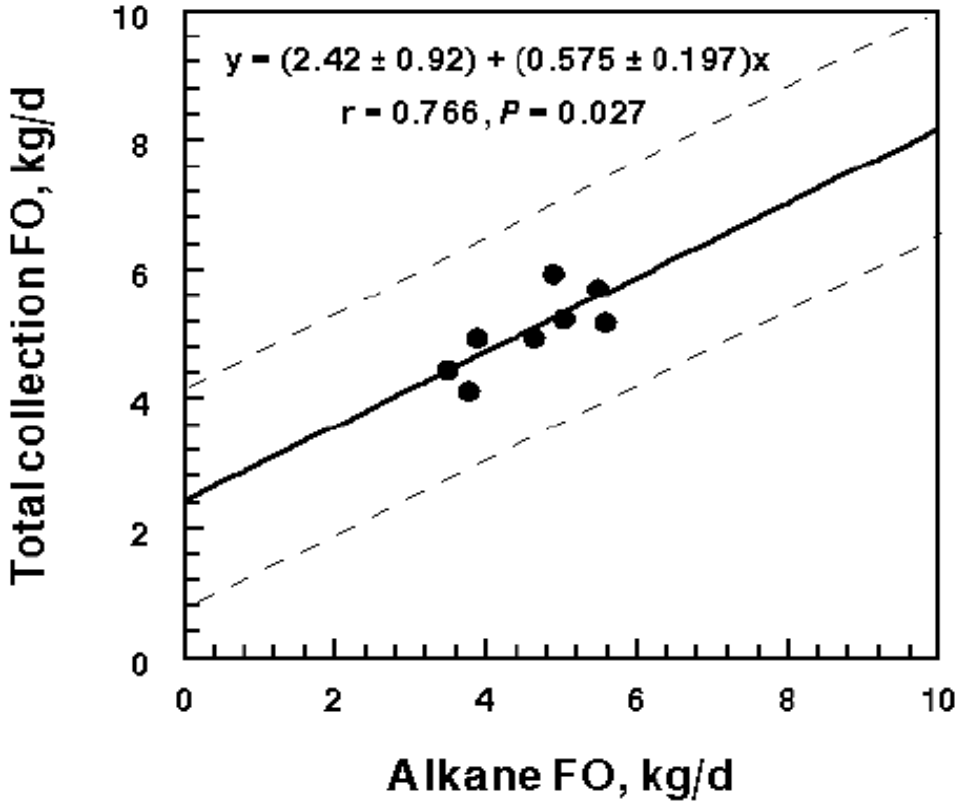

Figure 16. Calibration. FO_E prediction of FO_{TC} for the H diet (H).

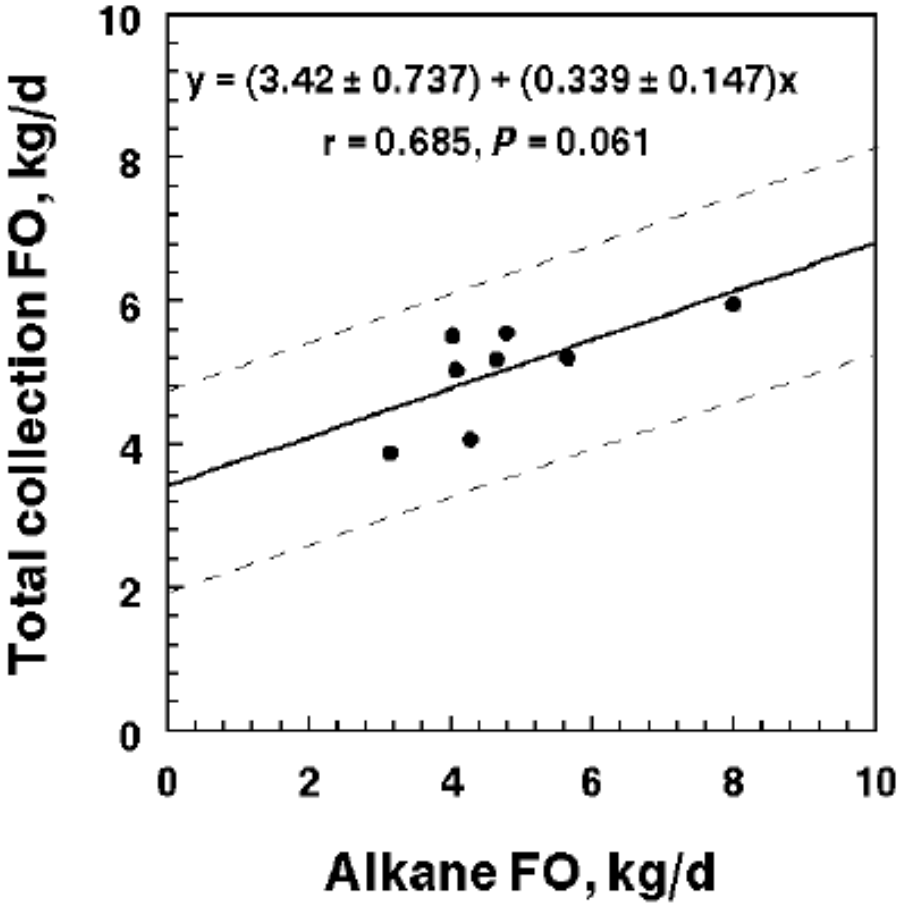

Figure 17. Calibration. FO_E prediction of FO_{TC} for the H and CBP (H+CBP).

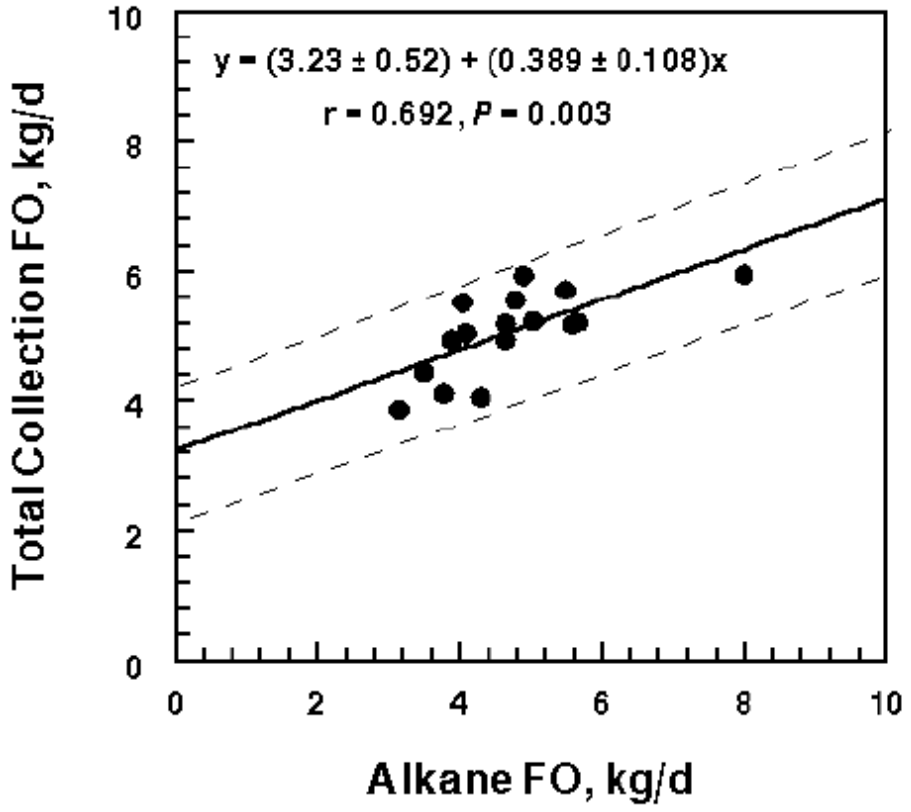

Figure 18. Calibration. FO_E prediction of FO_{TC} for both feeds (H, H + CBP).

Figure 19. Horse fitted with nappy (Equisan Corp., Australia) for total collection of feces.

Appendix table 1. Dietary treatments for horses offered hay (H) or hay and CBP (H + CBP).

	Diet	
Horse	Period 1[a]	Period 2[b]
1	H	H + CBP
2	H	H + CBP
3	H	H + CBP
4	H	H + CBP
5	H + CBP	Hay
6	H + CBP	Hay
7	H + CBP	Hay
8	H + CBP	Hay

[a] September 2000
[b] October 2000

Appendix table 2. Total collection dry matter intake (DMI_{TC}, kg/day), fecal output (FO_{TC}, kg/day) and dry matter digestibility (DMD_{TC}, %) for hay (H).

	H		
Horse	DMI_{TC}	FO_{TC}	DMD_{TC}
1	9.40	5.23	44.37
2	9.46	4.94	47.74
3	9.57	4.94	48.43
4	9.57	5.18	45.86
5	10.79	5.94	44.98
6	9.00	4.13	54.19
7	12.18	5.72	53.08
8	7.83	4.45	43.18
Mean ± SE[a]	9.72 ± 0.45	5.06 ± 0.21	47.73 ± 0.01

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

Appendix table 3. Total collection dry matter intake (DMI_{TC}, kg/day), fecal output (FO_{TC}, kg/day) and dry matter digestibility (DMD_{TC}, %) for the hay and CBP (H + CBP).

	H + CBP		
Horse	DMI_{TC}	FO_{TC}	DMD_{TC}
1	12.43	5.98	51.69
2	9.84	4.07	58.66
3	12.01	5.56	53.73
4	8.53	3.88	54.54
5	10.71	5.52	48.43
6	10.07	5.04	49.94
7	11.14	5.19	53.45
8	10.27	5.21	49.31
Mean ± SE	10.62 ± 0.43	5.05 ± 0.26	52.47 ± 0.01

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

Appendix table 4. Alkane concentrations (mg/kg, DM) in the hay (H) and hay and cereal by-product (H + CBP).

	Alkanes				
Diet	C25	C27	C29	C31	C33
Hay					
Mean ± SE[a]	12.70 ± 0.8	21.65 ± 0.25	53.15 ± 1.05	161.60 ± 2.4	28.75 ± 1.05
CBP					
Mean ± SE	9.92 ± 0.99	9.16 ± 0.18	31.35 ± 0.45	22.0 ± 1.00	0.00 ± 0.00

[a] Standard error of the mean (n = 2)

Appendix table 5. Nutrient composition on a DM basis of the cereal by-product (CBP).

	CBP	
Components	Mean	SE
Crude Protein, %	17.45	0.05
Crude Fat, %	22.05	0.05
Acid Detergent Fiber, %	13.30	0.40
Neutral Detergent Fiber, %	29.00	1.30
Non-Structural Carbohydrates, %	16.85	1.35
Ash, %	14.68	0.11
TDN, %	89.50	0.50
DE, Mcal/lb	1.52	0.01
Calcium, %	3.00	0.04
Phosphorous, %	1.89	0.18
Magnesium, %	1.07	0.02
Potassium, %	0.81	0.01
Sodium, %	0.08	0.00
Iron, mg/kg	131.50	8.50
Zinc, mg.kg	112.00	3.00
Copper, mg/kg	11.50	0.50
Sulfur, %	0.19	0.01
Chloride Ion, %	0.04	0.00

[a] Analysis (AOAC, 1990) performed by Dairy One, Ithaca, N.Y. (n = 6)

Appendix table 6. Nutrient composition on a DM basis of hay (H)[a].

	H	
Components	Mean	SE
Crude Protein, %	14.1	0.3
Crude Fat, %	40.3	0.7
Acid Detergent Fiber, %	63.7	1.4
Neutral Detergent Fiber, %	11.5	1.6
Non-Structural Carbohydrates, %	2.3	0.1
Ash, %	8.6	0.5
TDN, %	0.81	0.01
DE, Mcal/lb	0.34	0.01
Calcium, %	0.22	0
Phosphorous, %	2.62	0.05
Magnesium, %	0.015	0.001
Potassium, %	0.158	0.012
Sodium, %	0.017	0.001
Iron, mg/kg	0.007	0.001
Zinc, mg.kg	0.041	0
Copper, mg/kg	0.002	0
Sulfur, %	0.2	0
Chloride Ion, %	1.19	0.1

[a] Analysis (AOAC, 1990) performed by Dairy One, Ithaca, N.Y. (n = 6)

Appendix table 7. Mean apparent digestibility ($D_{A,}$ %) for individual horses fed hay (H)[a].

	D_A H							
	Horse							
Components	1	2	3	4	5	6	7	8
CP	64.46	66.82	70.26	73.05	66.32	68.54	67.98	62.52
ADF	33.08	24.61	36.08	39.13	31.42	29.80	39.13	27.09
NDF	41.49	34.71	43.05	46.91	39.01	36.49	44.62	34.85
NSC	96.13	109.66	93.92	90.16	84.60	106.26	97.76	85.96
EE	21.17	33.88	28.92	34.75	35.89	36.56	36.59	12.24
ASH	54.50	38.37	42.69	55.06	51.92	47.82	53.71	43.48
Ca	49.19	50.41	54.73	62.18	47.92	47.78	52.11	46.41
P	9.60	8.55	9.32	14.99	8.09	18.08	7.93	-2.87
Mg	36.91	34.21	31.04	36.36	24.24	36.70	27.79	23.08
K	78.93	63.63	65.93	76.59	70.30	66.11	70.57	70.93
Na	-15.67	-118.01	2.13	-195.05	-90.97	-51.30	9.67	-294.87
Fe	-5.37	-386.53	-69.50	-159.18	-119.03	-168.42	-79.91	-164.10
Zn	-2.06	-70.29	2.13	-3.61	-53.19	-18.33	9.31	-19.46
Cu	17.52	0.52	18.79	26.34	-4.86	13.51	7.65	3.85
Mn	-10.03	-37.29	2.13	-5.74	-19.41	-3.16	6.56	-25.20
Mo	0.85	19.59	2.13	32.26	21.88	22.63	6.56	-12.82
S	60.34	59.79	63.30	63.87	55.73	63.89	60.29	54.87
Cl	97.50	91.44	90.13	93.55	92.56	93.50	94.11	91.94

[a]Analysis (AOAC, 1990) performed by Dairy One, Ithaca, NY (n = 8)

Appendix table 8. Mean apparent digestibility (D_A, %) for horses fed hay and CBP (H + CBP)[a].

	D_A H+CBP							
	Horse							
Components	1	2	3	4	5	6	7	8
CP	68.19	71.80	70.41	77.12	70.59	68.82	68.87	70.19
ADF	29.43	30.87	33.92	40.81	33.86	29.71	34.78	33.87
NDF	40.05	39.38	42.08	49.55	43.38	39.07	43.82	43.96
NSC	93.30	84.48	86.47	90.02	73.80	88.10	90.64	76.83
EE	65.58	68.46	64.33	77.41	67.84	69.06	76.82	78.53
ASH	49.02	35.41	36.51	42.48	45.76	40.22	41.69	38.42
Ca	37.89	39.88	40.44	43.60	43.54	43.45	39.38	35.33
P	13.79	7.08	6.97	15.48	13.17	17.22	15.38	5.11
Mg	28.93	27.63	24.91	33.27	28.53	33.47	30.66	22.76
K	72.18	66.19	62.02	69.59	72.35	64.47	64.78	68.60
Na	55.09	38.51	66.60	55.47	-21.16	11.69	2.48	-38.50
Fe	1.56	-129.18	-11.75	-173.20	-12.59	-70.97	-83.63	-106.61
Zn	11.80	1.73	14.77	-26.71	10.67	24.02	4.51	-19.90
Cu	11.33	16.16	25.78	39.07	45.49	24.34	28.36	37.04
Mn	12.63	-4.41	9.37	5.80	7.07	21.47	6.13	2.24
Mo	34.78	10.92	13.79	45.45	14.05	34.21	37.50	39.06
S	61.54	62.43	61.09	69.01	61.41	62.80	44.44	59.96
Cl	97.55	96.81	94.28	96.41	95.93	94.14	92.36	95.15

[a] Analysis (AOAC, 1990) performed by Dairy One, Ithaca, NY (n = 8)

Appendix table 9. Mean partial digestibility ($D_{p,}$ %)for individual horses with the addition of the cereal by-product (CBP)[a].

	D_P CBP								
	Horse								
Components	1	2	3	4	5	6	7	8	SE
CP	81.0	85.68	70.81	87.23	82.77	69.55	71.74	85.64	2.6
ADF	-17.57	96.47	12.10	56.51	60.04	28.82	-17.94	85.23	15.50
NDF	26.61	74.86	34.94	67.43	77.38	57.49	36.79	93.99	8.5
NSC	85.12	25.22	69.57	89.73	47.77	47.94	71.26	61.32	7.56
EE	85.22	80.91	76.61	91.10	79.62	80.05	93.57	95.78	2.56
ASH	35.39	29.42	24.46	19.79	33.00	25.77	13.56	31.02	2.56
Ca	24.92	30.05	27.58	28.13	39.35	39.65	25.63	27.85	2.06
P	16.99	6.17	5.56	15.76	16.41	.16.72	20.74	8.70	2.02
Mg	21.96	22.95	20.71	31.31	31.65	31.31	33.02	22.60	1.87
K	-20.61	94.83	19.86	-0.11	95.83	47.23	-10.13	49.76	15.95
Na	110.80	138.75	106.40	198.51	24.63	49.60	-2.85	80.19	22.93
Fe	36.81	936.13	218.67	-224.94	438.64	308.13	-101.44	65.42	128.60
Zn	20.74	39.54	21.17	-37.52	45.00	44.91	1.60	-20.07	10.88
Cu	-4.02	47.71	39.37	61.97	149.51	44.87	76.68	85.44	15.69
Mn	21.20	5.70	11.52	8.96	15.41	28.59	5.98	8.34	2.86
Mo	130.92	-9.07	39.70	72.56	-4.44	59.30	120.01	125.53	19.84
S	66.91	72.04	53.34	85.69	82.59	59.07	-22.30	77.65	12.21
Cl	103.07	649.30	505.82	358.76	451.01	156.02	-115.89	333.89	86.99

[a]Analysis (AOAC, 1990) performed by Dairy One, Ithaca, NY. (n = 8)

Appendix table 10. Mean horse weights (kg) for duration of study.

	Period 1 and Period 2
Horse	Mean weight ± SE[a] (kg)
1	549.00 ± 2.95
2	714.36 ± 2.99
3	614.36 ± 2.29
4	577.57 ± 2.52
5	559.79 ± 0.73
6	620.36 ± 1.81
7	545.71 ± 1.39
8	598.36 ± 2.62
Mean ± SE	597.5 ± 19.52

[a] Standard error of the mean (n = 6)

Appendix Table 11. List of abbreviations and their definitions.

Abbreviation	Meaning
ADF	Acid Detergent Fiber
AIA	Acid Insoluble Ash
C_F	Concentration of Marker in Feces
C_I	Concentration of Marker in Feed
CBP	Cereal By-Product
D_A	Apparent Digestibility
D_P	Partial Digestibility
DE	Digestible Energy
DMD	Dry Matter Digestibility
DMD_E	Alkane Estimated DMD
DMD_{TC}	Total Collection Estimated DMD
DMI	Dry Matter Intake
DMI_E	Alkane Estimated DMI
DMI_{TC}	Total Collection Estimated DMI
EE	Ether Extract
FO	Fecal Output
FO_E	Alkane Estimated FO
FO_{TC}	Total Collection Estimated FO
H	Hay Diet
H + CBP	Hay Plus Cereal By-Product Diet
M	Marker
NDF	Neutral Detergent Fiber
R	Recovery
TC	Total Collection

Vita

Bridgett McIntosh Byrd, daughter of Mr. Robert McIntosh and Mrs. Beverly McIntosh was born on February 7, 1975 in Stamford, New York. She graduated from South Kortright Central School in South Kortright, New York in 1993. She then attended Hollins College in Roanoke, Virginia, majoring in Biology and was a member of intercollegiate riding team. The author was awarded the Bachelor of Arts degree in May 1997. Following her undergraduate degree, she pursued graduate studies in the Department of Animal and Poultry Sciences at Virginia Polytechnic Institute and State University in Equine Nutrition.