Alkanes as Internal Markers to Estimate Digestibility in Horses

Amy L. Ordakowski

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David S. Kronfeld, Chairman
David S. Sklan
Joseph H. Herbein
Larry A. Lawrence

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(ABSTRACT)

Fecal recoveries of n-alkanes were determined in three digestion balance experiments consisting of two periods each. Each experiment compared two diets in a switch-back design using eight mature Thoroughbred geldings. Horses were randomly assigned to two groups of four and offered one of two mixed grass/legume hays (Diets 1 and 2) in Exp. 1, mixed grass/legume hay and one of two concentrates (Diets 3 and 4) in Exp. 2, and mixed grass/legume pasture (Diets 5 and 6) in Exp. 3. In Exp. 1 and 2, horses were housed in stalls and in Exp. 3, two horses from each diet were housed in stalls and two horses remained on pasture. Balance periods lasted 11 days with d 1 to d 7 consisting of a dietary accommodation period, followed by 4 days of total collection. Results indicated that fecal recoveries of odd-chain alkanes (C25 to C33) were less than 100 % and similar between chain lengths. Estimates of DMD (D_E) were similar to the total collection DMD (D_{TC}) for Diet 1 in Exp. 1, but underestimated D_{TC} for Diet 2 in Exp. 1 ($P < .05$) and Diets 3 and 4 in Exp. 2 ($P < .05$). For Diet 5 in Exp. 3, the D_E for stall-fed horses using C25 and C33 was similar to D_{TC}, whereas C27, C29, and C31 underestimated D_{TC} ($P < .05$). For pastured horses, the D_E using C29 and C31 were similar to D_{TC}, whereas C25, C27, and C33 underestimated D_{TC} ($P < .05$). For Diet 6 in Exp. 3, the D_E for stalled horses calculated using C25 was similar to the D_{TC}, whereas use of C27, C29, C31, and C33 underestimated D_{TC} ($P < .05$). For pastured horses, the D_E using C29 was similar to D_{TC}, whereas all other alkanes underestimated D_{TC} ($P < .05$). When D_E was adjusted (D_{A1}) using the mean recovery of each odd-chain alkane, D_{A1} was similar to D_{TC} for Diet 2 in Exp. 1, Diets 3 and 4 in Exp. 2, and stalled horses offered Diets 5 and 6 in Exp. 3. The D_{A1} using C25 underestimated D_{TC} for Diet 1 in Exp. 1 ($P < .05$). For pastured horses offered Diet 5, D_{A1} for C33 was not different from the D_{TC} estimate, whereas all other D_{A1} for n-alkanes overestimated

Abstract

D_{TC} ($P < .05$). For pastured horses offered Diet 6, D_{A1} for C29 and C31 overestimated, but were similar to the D_{TC}, whereas the D_{A1} for C33 underestimated D_{TC} and was similar to the D_{TC}. The D_{A1} for C25 and C27 overestimated D_{TC} ($P < .05$). When D_E was adjusted for the mean recovery of all n-alkanes (D_{A2}), all D_{A2} estimates for stalled horses in Exp. 1, 2, and 3 were similar. In pastured horses offered Diets 5 and 6 in Exp. 3, the D_{A2} overestimated D_{TC} ($P< .05$). These results suggest that accurate mean estimates of DMD can be obtained by adjusting for mean recovery of each odd-chain alkane in a specific diet.

(Keywords: alkanes, digestibility, internal marker, horse)

Abstract

Acknowledgments

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This thesis is dedicated to my sister:

Kelly Marie Dixon

(1977 - 1992)

Table of Contents

List of Tables

List of Figures

List of Appendix Tables

Introduction

Individual measurements of dry matter intake (DMI), dry matter digestibility (DMD), and fecal output (FO) are essential components needed for the evaluation of the nutrition of grazing animals. Horses at maintenance meet most or all of their dietary needs from pasture, however, horses with increased nutritional demands due to exercise, growth, or pregnancy may require the addition of dietary supplements (hay and concentrate). To effectively incorporate a supplement into the diet, its effect on pasture intake and digestibility should be determined. Estimates of FO are also useful for evaluating the effects of the grazing animal (e.g. nitrogen and phosphorous excretion) on the environment.

Since DMI, DMD, and FO of the grazing animal are difficult to measure directly in the field, indirect methods using external and internal markers have been developed (Sutton et al., 1977; Owens and Hanson, 1992; Mayes et al., 1995). An external marker is a substance administered to the animal and used to estimate FO, whereas an internal marker is an inherent substance in the feed, usually part of the cell-wall fraction, which can be used to estimate DMD. The DMI of pasture can then be estimated from the DMD and FO estimates. For this method to be most accurate, markers should be chemically discrete entities, indigestible and unabsorbable in the digestive system, pass through the gut at a constant rate, and be physically similar to or intimately associated with the material it is to mark (Maynard et al., 1979; Owens and Hanson, 1992).

A new marker technique using plant wax constituents known as alkanes has received much attention for it's use to estimate pasture DMI in ruminants (Mayes et al., 1986; Dove and Mayes, 1991; Mayes et al., 1995). Alkanes are chemically discrete entities, abundant in the cuticular wax of pasture species, and readily analyzed using gas chromatography (Mayes and Lamb, 1984). In addition to these advantages, no marker preparation is required so that costs are reduced. Although alkanes were considered less "ideal" for estimating DMD in ruminants due to

increasing recovery with increasing chain length (Mayes and Lamb, 1983), preliminary studies suggests that alkanes behave differently in the gut of non-ruminants (Dove and Mayes, 1995; O'Keefe and McMeniman, 1998). No studies have been reported using odd-chain alkanes as internal markers to estimate DMD in the horse.

The objective of this study is to evaluate the use of naturally occurring odd-chain alkanes as internal markers to estimate DMD of forage and forage-plus-concentrate diets in stall-fed horses, and to test this method in horses under grazing conditions.

Review of Literature

Individual measurements of dry matter intake (DMI), dry matter digestibility (DMD), and fecal output (FO) are essential components needed in the evaluation of the nutrition of grazing animals. Horses at maintenance meet most or all of their dietary needs from pasture, however, horses with increased nutritional demands due to exercise, growth, or pregnancy may require the addition of dietary supplements (hay and concentrate). To correctly incorporate a supplement into the diet, its effect on pasture intake and digestibility should be determined. Estimates of FO are also useful for evaluating the effects of the grazing animal (e.g. nitrogen and phosphorous excretion) on the environment.

The common method for estimating DMI of pasture by horses under field conditions is the "by difference" method (Pagan, 1995; Kronfeld, 1998). This method accepts estimates of mean digestible energy (DE) intake based on the horses body weight and physiological condition (NRC, 1989) and subtracts estimates of DE of supplementary concentrates and/or hay. Any difference in DE is assumed to be provided by pasture DMI consumed by the horse. Too often, negative values of DMI have been calculated, thus this method is considered to be inaccurate and unreliable. More accurate and precise methods for estimating DMI of grazing animals involve indirect methods using markers (Kotb and Luckey, 1972), such as n-alkanes (Dove and Mayes, 1991; Mayes et al., 1995).

Traditionally, estimates of FO and apparent DMD have been determined by the total collection (TC) method (Sutton et al., 1977; Holechek et al., 1986). The TC method involves weighing food offered and food refused (orts), and total collection of feces either manually or by collection harnesses. The DMD is calculated using the actual DMI and FO values. Overestimation of DMD estimations can occur if feces and orts are under collected. The TC method is both labor intensive and expensive, which usually limits the number of animals that can be used. In addition, animals are usually confined in stalls, which may alter feeding behavior

and thereby lead to errors in DMD estimates. Although the TC method is not without its shortcomings, it usually serves as the standard for comparison in studies designed to validate other methods of estimating DMI, DMD, and FO.

An *in vitro* method provides DMD estimates of a specific forage by fermentation with gut microorganisms in a buffered medium under controlled anaerobic conditions, temperature, and pH (Minson, 1990). In ruminants, rumen microorganisms are used in a 48 hour digestion period, followed by a 24 hour digestion with pepsin-HCL (Tilley and Terry, 1963). To estimate DMD of equine feeds, an in vitro technique adapted from Tilley and Terry (1963) used cecal fluid obtained from horses instead of rumen liquor (Trevor-Jones et al., 1991). The *in vitro* methods apply a single DMD value of a forage to all experimental animals which does not account for differences in digestive efficiency within and between animals due to physiological state or level of intake (Orton et al., 1985a; Martin et al., 1989).

Of the methods used to used to predict DMD, regressions relating DMD to crude fiber, neutral detergent fiber, and acid detergent fiber of a specific forage are the most common (Minson, 1990). In addition, other methods of estimating DMD of a forage have included Near Infrared Reflectance Spectrophotometry (Andrieu et al., 1995), regressions relating forage digestibility to fecal composition, and indigestible internal markers (Minson, 1990). Of the previously mentioned methods, the use of indigestible internal markers has the most potential for accurately determining the DMD of forages fed to individual animals.

Markers in Equine Nutrition Studies

Since DMI, DMD, and FO of the grazing animal are difficult to measure directly in the field, indirect methods using external and internal markers have been developed (Sutton et al., 1977; Owens and Hanson, 1992; Mayes et al., 1995). An external marker is a substance administered to the animal and used to estimate FO, whereas an internal marker is a substance in the feed, usually part of the cell-wall fraction, which can be used to estimate DMD. The DMI of

pasture can then be estimated from the DMD and FO estimates. For this method to be most accurate, markers should be chemically discrete entities, indigestible and unabsorbable in the digestive system, pass through the gut at a constant rate, and be physically similar to or intimately associated with the material it is to mark (Maynard et al., 1979; Owens and Hanson, 1992). Markers were initially used to relate periods of feed intake to corresponding periods of FO. Subsequently, dilution calculations were applied to estimate transit time and FO (Merchen, 1988, Holland et al., 1998).

External markers. Chromium oxide (Cr_2O_3) is the most commonly used external marker to estimate FO (Schurg, 1981; Barbisan et al., 1993; Cuddeford and Hughes, 1990) and has also been used to determine fecal kinetics in the horse (Orton et al., 1985a; Holland et al., 1998). Problems associated with the use of Cr_2O_3 include cyclic fluctuations in excretion of the marker (Haenlein et al., 1966; Cuddeford and Hughes, 1990; Holland et al., 1998), lack of association with either the particulate or liquid component of the ingesta (Haenlein et al., 1966; Prigge et al., 1981), and the large dose required to attain accurate laboratory analysis. The rare earth element, Ytterbium (Yb), is another external marker that has been used to estimate FO by grazing horses (Meacham et al., 1986; Moffitt et al., 1987; Martin et al., 1989). However, these studies did not compare FO estimates from Yb to total collection of feces. Therefore, validation studies using Yb to estimate FO must be conducted to merit its use in future studies. Ytterbium has also been used to study the fecal kinetics of horses that had extensive large-colon resections compared to horses that did not have the resection (Bertone et al., 1989).

Internal markers. The use of acid insoluble ash (AIA) as an internal maker is the most commonly used method to estimate DMD in horses. In studies with stall-fed horses, AIA consistently underestimated DMD compared to total collection methods, however differences were not significant (Sutton et al., 1977; Orton et al., 1985a; Cuddeford and Hughes, 1990). The use of AIA to estimate DMD, hence digestible energy and digestible nitrogen, has also been used in grazing pregnant and lactating mares (Martin et al., 1989). Although lignin was one of the first plant components investigated in equine digestion studies, using lignin as an internal marker

results in underestimates of DMD between 12 to18 % (Schurg, 1981). Therefore, lignin is considered inaccurate and unsuitable as an internal marker. Another method using indigestible neutral detergent fiber (INDF) has been used to estimate apparent DMD of tall fescue (Meacham et al., 1986) and tall fescue and orchardgrass/clover pasture (Moffit et al., 1987) grazed by young light breed horses. However, these studies did not compare total collection of feces to DMD estimates using INDF. Therefore, validation studies comparing total collection and INDF estimates need to be conducted to justify its use in future studies.

Double Marker Methods. The potential value of using double marker methods in equine nutrition research is to accurately and precisely estimate DMI by the grazing horse. Chromium oxide and AIA were used as external and internal markers, respectively, to estimate pasture DMI of pregnant and lactating mares grazing mixed grass/legume pasture (Martin et al., 1989). Mares were divided by physiologic status (pregnant or lactating) and diet (supplemented or non-supplemented). The DMI determined from estimates of DMD by AIA and estimates of FO by Cr_2O_3 indicated that supplemented mares consumed less pasture than the non-supplemented mares (7.6 and 9.0 kg, respectively, $P < .05$) and lactating mares consumed more pasture than pregnant mares (9.6 and 7.0 kg, respectively, $P < .01$). The practical application of double markers under field conditions was used to show the ability of lactating mares to increase DMI intake to meet additional nutritional needs.

Although many internal and external markers have been tested in stalled and field conditions, nutritionist continue to investigate other potential substances that may provide more accurate and precise estimations of DMI, DMD, and FO.

Alkanes as Internal Markers

A new marker technique using plant wax constituents called alkanes has received much attention for its use to estimate pasture DMI in ruminants (Mayes et al., 1986a; Dove and Mayes, 1991; Mayes et al., 1995). Alkanes are chemically discrete entities, abundant in the cuticular

wax of pasture species, and readily analyzed using gas chromatography (Mayes and Lamb, 1984). In addition to these advantages, no marker preparation is required so that costs are reduced. Although alkanes were considered less "ideal" for estimating DMD in ruminants due to increasing recovery with increasing chain length (Mayes and Lamb, 1984), preliminary studies suggests that alkanes may behave differently in the gut of non-ruminants (Dove and Mayes, 1995; O'Keefe and McMeniman, 1998). The higher losses of the shorter chain n-alkanes (i.e. C25) compared to the longer chain n-alkanes (i.e. C35) may be ascribed to ruminal digestion. Therefore, this pattern may not be observed in the horse.

Characteristics of Alkanes

Alkanes are a subgroup of hydrocarbons that exist in small amounts in herbivore diets. Specifically, alkanes are composed of carbons in a straight chain saturated with hydrogen at each bond position and are located in the cuticular wax on the surface of plant cells (Malossini et al., 1990; Dove and Mayes, 1991; Laredo et al., 1991). Over 90% of the total alkane concentration in plant wax consists of odd carbon chain lengths in the range of C21 to C37 (Tulloch, 1976). Of the odd-chain alkanes, C29, C31, and C33 usually have the highest concentrations (Malossini et al., 1990; Dove and Mayes, 1991; Mayes et al., 1995). Higher concentrations of odd-chain alkanes are synthesized by decarboxylation of fatty acids of even carbon number (Barrowman et al., 1989). Even-chain alkanes also exist in plant waxes, but only make up approximately 3 to 6 % of the total alkane content. The lower concentration of even-chain alkanes has been suggested to limit the precision and accuracy of their analysis (Mayes et al., 1986a; Malossini et al., 1990).

Concentrations of alkanes with different chain lengths vary within individual plant parts (i.e. stem vs leaf) and between plant species (Malossini et al., 1990; Dove and Mayes, 1991; Dove et al., 1996). In a study evaluating odd and even-chain alkane concentrations (C27 to C35) in various grasses and legume species, similar patterns of alkane concentrations were observed within each grass and legume species (Malossini et al., 1990). Also, differences in alkane concentrations occurred between grass and legume species with legumes usually having higher

concentrations of alkanes compared to grasses. Of the odd-chain alkanes present, C29 and C31 had the highest concentrations followed by C27 and C33. In addition, alkane concentrations were observed to differ between individual plant parts of grasses and legumes including stem, leaf, and flower (Dove et al., 1996). Due to the variation of alkane concentrations between species and within individual plants, accurate analysis of forage offered to animals is essential.

Digestion and synthesis. Disappearance and synthesis of dosed even-chain alkanes (C28, C32 and C36) and odd-chain alkanes (C25 to C35) were examined in eight 13-month-old sheep fed freshly cut perennial ryegrass (Mayes et al., 1988). Animals were fitted with a rumen fistula and T-piece cannulae in the duodenum (proximal to the bile and pancreatic ducts) and in the terminal ileum. For 18 days, sheep were dosed with even chain alkanes and given continuous intraruminal infusions of ^{103}Ru-phenanthroline and ^{51}Cr-EDTA as dual-phase digesta flow markers. The greatest disappearance of alkanes occurred in the small intestine, with results more consistent for endogenous odd-chain alkanes than for exogenous or dosed C28, C32, and C36. This difference may have been due to the fact that upon centrifugation of the contents collected, over 95 % of the odd-chain alkanes were precipitated and therefore associated with the particulate phase, while 30 to 40 % of the even-chain alkanes remained in the supernatant. Also, synthesis of alkanes was examined in four of the sheep that received continuous intraruminal infusions of [^{14}C]-sodium acetate. There was a loss of 5.03 % of the infused ^{14}C-acetate in the feces, while only .00042 % appeared in the fecal alkane fraction. The results of this study indicate that disappearance of alkanes in the gut of ruminants is most likely due to absorption in the small intestine and that negligible synthesis of alkanes occurred in the digestive tract.

Another study investigated the site of loss of dosed C32 in the gut of three cows fitted with ruminal and T-type duodenal cannulas and fed a mixed hay and concentrate diet (Ohajuruka et al., 1991). In the first period, cows received the dosed C32 via the duodenal cannula and in Period 2, the cows received the dosed C32 via the rumen cannula. Based on the fecal recovery of C32 obtained from total collection methods, the recovery of the dosed marker was lower with ruminal dosing than the duodenal dosing. These results suggest ruminal loss of the marker,

which is in contrast to previous reports of higher losses of alkanes occurring in the small intestine.

Absorption and metabolism. Absorption and metabolism of odd and even-chain alkanes was also examined in the digestive tract of two 13 month old Blackface wethers fed freshly cut perennial ryegrass (Mayes et al., 1988). Animals were dosed twice daily with C32 for 5 days, followed by dosing of [^{14}C]-C32 alkane for 3 days. During the last 36 hours of the radioactive dosing period, continuous intravenous infusions of [^{13}C]-Na_2CO_3 were given to measure CO_2 production rate and the proportion of the ^{14}C-C32 alkane oxidized. Jugular blood samples were taken during the last 9 hours of infusion. Animals were sacrificed, the gut contents removed, and tissue sample taken for ^{14}C analysis. Although 9.3 % of ^{14}C from the [^{14}C]-C32 alkanes was not recovered from the gut, only 1.6 % of the ^{14}C from the [^{14}C]-C32 alkanes was recovered in CO_2. Results of this study indicate that about one-sixth of the absorbed alkanes is oxidized in sheep. Long chain alkanes can be metabolized to the corresponding fatty acids, which will then enter metabolic pathways (Barrowman et al., 1989).

Fecal Recovery. Initially, odd-chain alkanes (C25 to C35) in herbage were investigated as potential internal markers for estimating DMD in ruminants based on their chemically discrete nature and relative ease of analysis using gas chromatography (Mayes and Lamb, 1984). However, in lambs fed ryegrass and white clover mixed herbage, incomplete fecal recovery (fraction of the ingested alkane recovered in the feces) of C25, C27, C29, C31, C33, and C35 was observed. Fecal recovery of odd-chain alkanes increased as the carbon chain length increased, with C25 having the lowest mean recovery of 19.5 % and C35 having the highest mean recovery of 97.5 %. Because accurate estimations of DMD require 100 % recovery, it was suggested that the longer chain alkanes may be useful as internal markers for estimating DMD in grazing ruminants. Similar patterns of increasing recovery with increasing chain length have been observed in other ruminant studies (Mayes et al., 1986a; Mayes et al., 1986b; Bechet and Tulliez, 1992). In sheep fed medic cultivars, a quadratic relationship ($r^2 = 0.985$) between chain length and recovery was observed with peak recovery of 90 % for C32 (Casson et al., 1990). In

the same study, fecal recoveries of n-alkanes were observed to vary with the pasture type, indicating that the use of n-alkanes in the field would require the need for calibration for each pasture plant/species used. In addition to accuracy (difference between observed recovery and 100 %), an estimate of precision (e.g. SE) is needed for each pasture type.

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). Also, in seven horses fed seven diets each containing different proportions of oaten chaff, alfalfa chaff, proprietary horse pellets, and cottonseed meal, recovery of n-alkanes of plant origins were observed to be higher than those in ruminants (O'Keefe and McMeniman, 1998). Fecal recoveries of odd-chain alkanes with carbon chain length C25 to C33 were in the range of 82 to 99 %. In addition, no trend was observed for higher fecal n-alkane recovery with increasing carbon chain length. The fecal recoveries reported in this study are mean values for the seven diets offered to horses. Because fecal recovery has been shown to vary with the diet offered (Casson et al., 1990), reports of mean fecal recovery for the individual diets may prove more useful.

Estimates of Digestibility using Alkanes

Estimates of DMD are not only important for assessing the nutrient value of forage and/or supplements offered to horses, but also to describe the digestive efficiency of individual horses. Studies evaluating the use of n-alkanes to estimate DMD of diets fed to horses are few (Mayes et al., 1995). Based on previous studies in ruminants (Mayes et al., 1986a; Mayes et al., 1986b; Bechet and Tulliez, 1992) and horses (O'Keefe and McMeniman, 1998), corrections for incomplete fecal recovery would be required if the natural alkanes were to be used on their own as markers to estimate DMD. Dove et al. (1990) compared estimates of DMI determined from Cr_2O_3 estimates of FO and DMD estimates from either pentatriacontane (C35) or *in vitro* methods in lactating sheep grazing perennial ryegrass. Estimates of DMD using C35 were corrected, assuming the fecal recovery of C35 to be 95 %. The C35 based digestibilities

consistently overestimated *in vitro* estimates at 14 (79.8 ± 5.7 vs 82.9 ± 2.0 %), 28 (77.1 ± 1.3 vs 81.5 ± .6 %), 42 (76.8 p 1.0 vs 86.0 ± .9 %), and 85 d (76.0 ± .4 vs 81.6 ± .6 %) of lactation, and there was a difference ($P < .01$) in DMI estimates at 28, 42, and 85d of lactation. The C35 based digestibility estimates corrected for incomplete recovery were more consistent and similar to mean values reported in the Agricultural Research Council (1984) and were therefore considered more accurate than the *in vitro* estimates. Although C35 was useful in this instance, its relatively low concentrations in temperate pasture species may limit the accuracy of estimates when using these species (Malossini et al., 1990). Shorter odd-chain alkanes (C27 to C33) which exist in higher concentrations in temperate pasture species may yield accurate estimations of DMI of horses when corrected for incomplete recovery.

In a study comparing methods for estimating supplement intake and DMD in sheep, DMD was estimated *in vivo* by total collection methods, and was also estimated using the diet and fecal concentrations of indigestible acid detergent fiber (IADF), C31 and C33, and lignin (Dove and Coombe, 1992). Mean fecal recovery of IADF and lignin were 97.4 ± 1.0 and 109.2 ± .7 %, respectively. Fecal recovery of C27 to C33 increased with increasing chain length in the range of 63.0 ± 1.3 to 87.2 ± 1.8 %. Both C31 and lignin based digestibilities overestimated *in vivo* estimates by 1.0 ($P > .05$) and 3.2 % ($P < .05$), respectively, while IADF underestimated DMD by approx. 1.7 % ($P > .05$). The C33 estimate of DMD was almost identical to the *in vivo* estimate. It should be noted that the C31 and C33 estimates were adjusted for incomplete recovery using previously reported mean recoveries (Dove and Mayes, 1991).

Future Uses of Alkanes in Equine Nutrition Studies

Botanical Composition. Because pasture differs in nutritive value between species and within individual plant parts, it would be beneficial to equine nutritionists to not only assess how much a horse is consuming while grazing pasture, but also which specific plant and/or plant parts the horse is selectively grazing. Studies evaluating alkane concentrations in forages observed

differences in alkane concentration between species and within individual plant parts (Malossini et al., 1990; Dove and Mayes, 1991; Dove et al., 1992). The principle of using alkanes to estimate botanical composition remains in the number of species or plants parts under investigation and the number of n-alkanes in those components. Simultaneous equations have been used to estimate the botanical composition of forage mixtures using n-alkanes as markers (Dove, 1992). In order to achieve this, the number of equations were the same as the number of species under investigation and the number of n-alkanes chosen as markers. These procedures have been reviewed in several studies (Dove, 1992; Dove and Moore, 1995; Newman et al., 1995) and reviews (Dove and Mayes, 1991; Mayes et al., 1995; Dove and Mayes, 1996).

Pasture Intake. The estimation of pasture DMI using a dosed even-chain alkane and an inherent odd-chain alkane has shown much success in ruminants (Mayes et al., 1986a; Dove and Mayes, 1991; Bechet and Tulliez, 1992), but is not well advanced in non-ruminants. The double marker method first developed by Mayes et al. (1986a) uses an odd-chain alkane in herbage as an internal marker and an adjacent even-chain alkane dosed as an external marker (i.e. C31 and C32). The fecal and herbage concentration of the odd and even-chain alkanes, as well as the dose rate for the even chain alkanes are required to calculate pasture DMI. Fecal concentrations of both odd and even chain alkanes are expressed as a ratio and in effect, equal biases associated with their incomplete, but approximately equal recoveries cancel out. Several ruminant studies employing the double marker method found the most accurate estimates of DMI using C32 and C33 alkanes (Mayes et al., 1984; Bechet and Tulliez; Dove et al., 1995).

In theory, the double marker method for estimating pasture DMI in horses should give accurate estimations, provided the even and odd-chain alkanes used have similar fecal recoveries. The double marker alkane method for estimating intake in horses and ponies and in pigs has been examined in separate indoor experiments (Mayes et al., 1995). In both studies, animals were dosed twice daily with the C32 alkane and results indicate that accurate estimates of DMI intake were obtained using the C32:C33 alkane pair. In addition, fecal recovery did not increase substantially with increasing chain length in either study (Mayes et al., 1995) which is similar to

recent observation in horses (O'Keefe and McMeniman, 1998). Similar recoveries between chain lengths would facilitate estimates of pasture intake. Instead of the need for adjacent dosed and natural alkane pairs for effective intake estimation (i.e. C32 and C33), accurate intake estimates may be obtained using non-adjacent alkane pairs provided their recoveries are similar. The only study that reported values of fecal recovery of dosed even-chain alkanes in horses observed recoveries of C26, C28, C30, C32, and C36 to be 77, 70, 99, 73, and 75 %, respectively (O'Keefe and McMeniman, 1998). As mentioned earlier, the fecal recoveries reported in this study are mean values of fecal recovery for horses offered different proportions of oaten chaff, alfalfa chaff, proprietary horse pellets, and cottonseed meal. Because fecal recovery has been shown to vary with the diet offered (Casson et al., 1990), reports of mean fecal recovery for the individual diets may prove more useful.

The primary limitation of using the dosed even-chain alkanes in DMI studies is their expense. Standard kits are available and provide alkanes in the range of C23 to C30 and then even-chain alkanes up to C50 (HSH-15, 50 g, $ 249.15, Sigma Chemical, St. Louis, MO). It is possible to extract alkanes from plant waxes, but this requires a laboratory that has appropriate equipment and abundant technical assistance. Less expensive methods of estimating DMI are being tested in our laboratory by combining DMD estimates by endogenous alkanes and FO estimates by Cr_2O_3 kinetics.

Pasture and Supplement Intake. Horses at maintenance meet most or all of their dietary needs from pasture, however, horses with increased nutritional needs for exercise, growth, or pregnancy may require the addition of dietary supplements (hay and concentrate). The potential exists, for the alkane double marker method to estimate DMI of both pasture and supplement of individual horses in group-fed conditions. If the concentration of n-alkanes in the pasture and supplement, as well as the concentration of alkanes in the feces are determined, simultaneous equations using two n-alkanes for two unknowns (pasture and supplement) should yield accurate estimations of DMI for each. Dry matter intake estimates of pasture and supplement by individual horses in group-fed conditions would allow the animal to be used as the statistical

unit. Often, group-fed horses are housed in two or three paddocks, limiting the degrees of freedom to 1 or 2, respectively. Allowing the horse to be considered the experimental unit would increase the degrees of freedom, thereby increasing the statistical power.

Objectives

The general objective of this study was to evaluate the use of naturally occurring odd-chain alkanes as internal markers to estimate dry matter digestibility of forage and forage-plus-concentrate diets offered to horses. More specific aims of the study included: 1) Determination of fecal recovery of odd-chain alkanes in hay (orchardgrass/alfalfa vs tall fescue/alfalfa), hay (orchardgrass/alfalfa)-plus-concentrate (Fat and Fiber vs Sugar and Starch), and pasture (bluegrass/white clover vs tall fescue/alfalfa) offered to horses in stalls; 2) Comparison of digestibility estimates of hay and hay-plus-concentrate diets fed to horses in stalls; 3) Comparison of digestibility estimates of pasture offered to stalled horses and horses grazing on pasture, to validate use of alkanes under field conditions.

Materials and Methods

Fecal recoveries of n-alkanes were determined in three digestion balance experiments consisting of two periods each. Each experiment compared two diets in a switch-back design using eight mature Thoroughbred geldings at maintenance. Horses were between the ages of 6 and 12 years and weighed 551 ± 63 kg (mean ± SE), 548 ± 62 kg , 559 ± 56 kg, for Exp. 1, 2, and 3, respectively. The protocol was approved by an Institutional Animal Care and Use Committee.

Dietary Treatments

Experiment 1. Eight horses were randomly assigned to two groups of four horses each. One group received tall fescue (*Festuca arundinacea*) and alfalfa (*Medicago sativa*) mixed hay (Diet 1) and the other group received orchardgrass (*Dactylis glomerata*) and alfalfa mixed hay (Diet 2) with a switch of diets between periods (Appendix table 1). All horses received their experimental diet while on pasture two weeks prior to the start of each period. Hays were second cuttings harvested in late June 1997 from the Virginia Tech Middleburg Agricultural Research and Extension Center. Nutrient composition (Dairy One, Ithaca, NY) of hays is shown in Appendix table 2. Each horse was initially fed diets at 2% of their body weight (BW) with rations adjusted weekly to maintain constant BW.

Experiment 2. Eight horses were randomly assigned to two groups of four horses each. One group received orchardgrass (*Dactylis glomerata*) and alfalfa mixed hay and fat and fiber (FF) concentrate (Diet 3) and one group received orchardgrass/alfalfa hay and starch and sugar (SS) concentrate (Diet 4), with a switch of diets between periods (Appendix table 3). All horses received their experimental diet while on pasture two weeks prior to the start of each period. Nutrient composition (Dairy One, Ithaca, NY) of hay and concentrates is shown in Appendix table 4. The SS was a commercial concentrate high in sugar and starch (Omolene 200, Purina Mills, Inc., St. Louis, MO) and the FF concentrate was a corn oil and fiber based concentrate (Appendix table 5). Hay was from the same cutting used in Experiment 1. Initially, hay was fed

to meet 50% and concentrate was fed to meet 50% of the daily energy requirement for horses at stall maintenance (NRC, 1989); however, hay intake was doubled on d 3 of dietary accommodation to eliminate coprophagy.

Experiment 3. Eight horses were randomly assigned to two groups of four and placed on either tall fescue (*Festuca arundinacea*) and alfalfa (*Medicago sativa*) mixed pasture (Diet 5) or kentucky bluegrass (*Poa pratensis*) and white clover (*Trifolium repens*) mixed pasture (Diet 6). Both 3.24 ha pastures were established in 1993 at the Middleburg Agricultural Research and Extension Center. Nutrient composition (Dairy One, Ithaca, NY) of pastures used is shown in Appendix table 6. Horses were placed on the pastures approximately two weeks before each period began to allow for dietary accommodation. On d 1 of each period, two horses from each pasture were placed in 4m x 4m individual box stalls and offered their respective pasture. Horses in stalls were fed clipped whole forage from their respective pastures in small frequent meals four times daily (0700, 1300, 1900, and 2200) to simulate grazing. Clipped pasture was initially offered at 10% of their BW with rations adjusted during the dietary accommodation period so that intake was not limited. Forage was clipped fresh every morning using a Gravely mower (Model 5665, Gravely International, Brillion, WI) and offered at the afternoon, evening, and subsequent morning feeding. Dietary treatments were switched between periods and are shown in Appendix table 7. Pastures were sampled during each period by harvesting strips (8 x 8 cm at 2.5 cm cutting height; one strip per .4 ha) to sort for botanical composition to allow alkane concentrations of individual species to be determined.

Experimental Procedures

Experiment 1 and 2. Exp. 1 and 2 consisted of two digestion balance periods conducted in February 1997 and March 1997 (Exp. 1) and in mid-April to mid-May 1997 and July 1997 (Exp. 2). Each period lasted 12 days. On d 1, horses were placed in 4m x 4m individual box stalls. Day 1 to d 7 consisted of a dietary accommodation period, followed by four days of total collection (d 8 to d 11). Hay was weighed, offered in a net, and the orts collected each morning and weighed. Samples of hay (Exp. 1) and hay and concentrate (Exp. 2) were collected every

other day from d 2 to 12. From d 8 to d 11, fecal grab samples were collected three times a day (0700, 1500, and 2300) and combined daily for each horse. Also, collection of feces was performed every 2 hours and total fecal output was recorded. Water was freely accessible and intake recorded daily.

Experiment 3. Two digestion balance periods were conducted in June 1997 and August 1997. Day 1 to d 7 consisted of a dietary accommodation period. From d 8 to d 11, fecal samples from stalled horses and fecal grab samples in pastured horses were taken from each horse at 0700, 1300, and 1900 and combined daily. Collection of feces from stalled horses was performed every 2 hours and total fecal output recorded daily from d 8 to d 11. Samples of pasture fed to stalled horses were collected daily from d 8 to d 11. In addition, a separate investigator conducted an adjacent study, dosing chromium three times daily to stalled and pastured horses to evaluated its use as an external marker to estimate FO. Estimates of FO by chromium and DMD by alkanes can then be used to estimate DMI for horses remaining on pasture.

N-Alkane Analyses

Hay, concentrate, pasture, botanical composition, and fecal samples were dried in a forced air oven and ground through a .5mm screen Cyclone Mill (Model 3010, UDY Corp., Fort Collins, CO). Ground hay and fecal samples collected from d 8 to d 11 were composited, mixed thoroughly, and two subsamples taken for duplicate alkane analysis. N-alkane content in hay, concentrate, pasture and feces was determined using gas chromatography (Model 5890A, Hewlett Packard, Wilmington, DE) according to the methods of Mayes et al. (1986) with minor modifications. A sample of feces (.1 g) or hay, concentrate, and pasture (.3 g) was placed in a Pyrex tube (20ml) fitted with a screw cap prior to the addition of 0.1008 mg of C34 (tetratriacontane) internal standard (Sigma Chemical, St. Louis, MO) and 7 ml of 10% ethanolic KOH (Sigma Chemical, St. Louis, MO). Tube contents were mixed thoroughly, placed in a water bath (90°C) for 3 hours, and mixed thoroughly every 30 min. After cooling of samples, 7 ml distilled water and 7 ml of heptane (Sigma Chemical, St. Louis, MO) were added and tube

contents were well mixed. The organic extract was removed, applied to a column consisting of silica gel (Sigma Chemical, St. Louis, MO) contained in disposable Oxford pipet tips (200 µl) with glass wool stoppers, and the effluent collected in 20 ml scintillation vials. Approximately 10 ml of heptane was used to rinse the column. The total eluent was allowed to evaporate over night in a fume hood. The dried sample was re-dissolved with 1ml heptane before injection of .5 µl onto a 30 m x .52 mm x 1.5 µm capillary column (Supelco Inc., Bellefonte, PA) in the gas chromatograph fitted with a flame ionization detector. The chromatograph column oven temperature was programmed (240°C for 4 min; 3°C/min to 288°C; 2°C/ min to 298°C) and the carrier gas, helium, had a flow rate of approximately 9 – 9.25 ml/min. The area under the peak for each n-alkane was determined using an integrator (Model 3393A, Hewlett Packard, Wilmington, DE). The identity of odd-chain alkanes (C25 to C33) was determined from their retention times relative to known standards, whereas peak areas were converted to amounts of alkane by reference to the internal standard.

Calculations

Experiments 1 and 3. Total collection DMD (D_{TC}, %) of each diet offered to stalled horses was calculated using mean diet intake (I, kg/d DM) and mean fecal output (O, kg/d DM) from d 8 to d 11:

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

Fecal recovery (R_1, %) of each odd-chain alkane (C25 to C33) were calculated for each stalled horse offered each diet using mean I, O, and n-alkane concentration in diets (C_I, mg/kg DM) and feces (C_O, mg/kg DM):

$$R_1 = ((O \bullet C_O) / (I \bullet C_I)) \bullet 100 \quad [2]$$

Also, the mean fecal recovery (R_2, %) of all odd-chain alkanes (C25 to C33) for each stalled horse offered each diet were calculated by plotting mean n-alkane output (O_M, mg/d) vs

mean n-alkane intake (I_M, mg/d) of all odd-chain alkanes. In addition, the Lucas plot (Pagan, 1998) was used to determine endogenous fecal losses (E_A, mg/d) of all odd-chain alkanes by plotting mean n-alkane absorption (A_M, mg/d) vs I_M.

Estimated DMD (D_E, %) of the diets for each horse in stalls and on pasture (Exp. 3) were calculated using C_I and C_O of odd-chain alkane length in the range of C25 to C33 as follows:

$$D_E = (1 - (C_I / C_O)) \bullet 100 \quad [3]$$

The D_E was adjusted (D_{A1}, %) for each horse in stalls and on pasture (Exp. 3) offered each diet using C_I, C_O, and R_1:

$$D_{A1} = (1 - (R_1 \bullet (C_I / C_O))) \bullet 100 \quad [4]$$

The D_E was also adjusted (D_{A2}, %) for each horse in stalls and on pasture (Exp. 3) offered each diet using C_I, C_O, and R_2:

$$D_{A2} = (1 - (R_2 \bullet (C_I / C_O))) \bullet 100 \quad [5]$$

Experiment 2. The D_{TC} of the diets were calculated for each horse using mean hay intake (I_H, kg DM/day), mean concentrate intake (I_C, kg DM/day), and O from d 8 to d 11:

$$D_{TC} = (1 - (O / (I_H + I_C)) \bullet 100 \quad [6]$$

Fecal recovery (R_1, %) of each odd-chain alkane (C25 to C33) was calculated using I_H, I_C, O, and n-alkane concentration in hay (C_H, mg/kg DM), concentrate (C_C, mg/kg DM), and C_O:

$$R_1 = ((O \bullet C_O) / ((I_H \bullet C_H) + (I_C \bullet C_C))) \bullet 100 \quad [7]$$

Also, the mean fecal recovery (R_2, %) of all odd-chain alkanes (C25 to C33) for each stalled horse offered each diet were calculated by plotting O_M vs I_M. In addition, the Lucas plot (Pagan, 1998) was used to determine E_A of all odd-chain alkanes by plotting A_M vs I_M.

Estimated DMD (D_E, %) of the diets for each horse were calculated using C_H, C_C, and C_O of odd-chain alkane length in the range of C25 to C33:

$$D_E = (1 - ((C_H + C_C) / C_O)) \bullet 100 \quad [8]$$

The D_E was adjusted (D_{A1}, %) for each stalled horse offered each diet using C_H, C_C, C_O, and R_1:

$$D_{A1} = (1 - (R_1 \bullet ((C_H + C_C) / C_O))) \bullet 100 \quad [9]$$

The D_E was also adjusted (D_{A2}, %) for each stalled horse offered each diet using C_I, C_O, and R_2:

$$D_{A2} = (1 - (R_2 \bullet (C_I / C_O))) \bullet 100 \quad [10]$$

Statistical Analyses

Data are summarized as means and standard error of the mean (SE) unless otherwise stated. The DMI, FO, fecal concentrations of n-alkanes, and alkane recoveries were analyzed separately using the GLM procedure of SAS (1989) with horse and diet in the model. N-alkane concentrations in diets were analyzed using the by GLM procedure of SAS (1989) with diet in

the model. Methods of estimating DMD of diets (total collection vs n-alkane methods) were compared using the GLM procedure of SAS (1989) with horse and method in the model. Tukey's studentized range test was used to test for differences in recovery of n-alkanes within diets and also to test for differences of DMD due to the method of estimation within diets. Fecal recovery of all n-alkanes and endogenous losses of n-alkanes were determined by linear regression using graphical software (Slide Write Plus, 1996).

Results and Discussion

Experiment 1

Balance data. The voluntary DMI, FO, and D_{TC} [equation 1] for each horse offered Diets 1 and 2 are shown in Appendix table 8. Mean DMI was similar (9.4 ± .61 vs 10.2 ± .54 kg/d, P = .95) and FO was similar (4.3 ± .29 vs 4.0 ± .30 kg/d, P = .96) for horses offered Diets 1 and 2, respectively. Mean D_{TC} of Diet 1 was lower than Diet 2 (54.1 ± 1.2 vs 60.9 ± 1.3 %, respectively, P = .015). The NDF content in Diet 1 was higher than Diet 2 (66.2 vs 62.6 %, respectively), which may have contributed to it's lower apparent DMD, since NDF is negatively correlated with DMD in ruminants (Minson, 1990). The D_{TC} of tall fescue/alfalfa mixed hay of Diet 1 (54.1 %) was in between total collection DMD estimates of 48.0 and 58.0 % observed for Arabians horses fed mid-bloom tall fescue and alfalfa hay, respectively (Crozier et al., 1997). In another study utilizing total collection methods, orchardgrass (early head stage) and alfalfa (one-quarter bloom stage) hay fed to geldings had apparent DMD of 50.0 and 60.8 %, respectively (Vander Noot and Gilbreath, 1970). The higher D_{TC} for the orchardgrass/alfalfa mixed hay of Diet 2 (60.9 %) observed in our study is most likely due to it being harvested at an earlier stage of maturity corresponding to a higher DMD (Darlington and Hershberger, 1968; Ball et al., 1996).

Marker data. Concentrations of n-alkanes (C25 to C33) did not differ between diets (Appendix table 9). The most abundant alkane was C31, followed by C29, C33, C27, and C25 in both diets. This pattern is in agreement with several studies evaluating n-alkane contents in specific grasses and legume species (Mayes et al., 1986; Malossini et al., 1990; Dove and Mayes, 1991). The concentrations of C27, C29, C31, and C33 observed in Diet 2 are lower than concentrations previously reported for alfalfa. Concentrations of C27 and C31 were lower, but C29 and C31 were higher than concentrations reported for orchardgrass (Malossini et al., 1990). Differences observed between our results and the results in the previous study are most likely due to our diets consisting of a mixture of grass and legume species and that alkane concentrations

vary with species maturity and within individual plant parts (Malossini et al., 1990; Dove, 1992). No previous data are available for n-alkane concentrations in tall fescue.

Fecal concentrations of n-alkanes for horses receiving Diets 1 and 2 are shown in Appendix table 10. Comparing Diets 1 and Diet 2, fecal concentrations were higher for C29 (93.3 ± 3.5 vs 121.6 ± 1.8 mg/kg, $P = .0001$) and C31 (218.1 ± 8.8 vs 257.0 ± 3.2 mg/kg, $P = .003$), but were lower for C25 (18.9 ± .90 vs 16.1 ± .50 mg/kg, $P = .039$) and C33 (33.1 ± .68 vs 27.5 ± .46 mg/kg, $P = .0001$), and no difference was observed for C27 (30.4 ± 1.1 vs 32.2 ± .75 mg/kg, $P = .19$).

Fecal recovery [2] of each odd-chain alkane (R_1) in Diets 1 and 2 are shown in Table 1 and 2, respectively. For Diet 1, R_1 of C25 was higher than the other n-alkanes ($P < .05$), and R_1 was similar for C27, C29, C31, and C33. The high R_1 of C25 in Diet 1 (103.7 %) is difficult to explain. For Diet 2, R_1 of was higher than the other n-alkanes ($P < .05$). The R_1 values of C25, C27, and C33 were similar, as well as those of C27, C31, and C33. In contrast to ruminant studies (Mayes and Lamb, 1983; Mayes et al., 1986; Dove and Mayes, 1991), fecal recovery of n-alkanes in both Diets 1 and 2 did not increase with increasing chain length. With the exception of C25 in Diet 1 and C29 in Diet 2, recoveries of C27, C29, C31, and C33 in both diets were slightly lower than fecal recoveries observed in horses fed seven diets each containing different proportions of oaten chaff, alfalfa chaff, proprietary horse pellets, and cottonseed meal (O'Keefe and McMeniman, 1998). Fecal recoveries observed in that study for C25, C27, C29, C31, and C33 were 86, 82, 85, 94, and 99 %, respectively. In addition, there was no trend reported for higher fecal recovery with increasing carbon chain length, which is in agreement with our results. The fecal recoveries of C25, C27, and C29 in this study were higher than recoveries reported for ruminants fed perennial ryegrass (Mayes et al., 1984; Mayes et al., 1986a; Mayes et al., 1986b). The higher recovery of the shorter chain alkanes in horses compared to sheep may be explained by a shorter retention time in the gut of non-ruminants compared to ruminants (Warner, 1981), which would decrease the time for metabolism of the alkanes.

Comparisons of fecal recovery between Diets 1 and 2 are shown in Figure 1. Fecal recovery of C25 in Diet 1 was higher than C25 recovery in Diet 2 (P = .015), but recovery of C27, C29, C31, and C33 were similar between Diet 1 and 2. In general, we observed similar recovery between Diets 1 and 2 which is in contrast to a study which observed fecal recoveries of n-alkanes to vary between three different legume pasture species fed to sheep (Casson et al., 1990).

The similar recoveries between chain lengths, with the exception of C25 in Diet 1 and C29 in Diet 2, would facilitate estimates of pasture intake using the double marker first proposed in non-ruminants (Mayes et al., 1986). Instead of the need for adjacent dosed and natural alkane pairs for effective intake estimation (ie. C32 and C33), our results suggest that accurate intake estimates may be obtained using non-adjacent odd and even-chain alkanes provided their recoveries are similar. In a study examining fecal recovery of naturally occurring even-chain and dosed even-chain alkanes in horses observed recoveries of C26, C28, C30, and dosed C32 and C36 to be 77, 70, 99, 73, and 75 %, respectively (O'Keefe and McMeniman, 1998). The n-alkane recoveries obtained in that study were averaged for seven diets offered to horses, each containing different proportions of oaten chaff, alfalfa chaff, proprietary horse pellets, and cottonseed meal. The low concentrations of the even-chain alkanes inherent in forage (Malossini et al., 1990) may have led to measurement inaccuracies for C26, C28, and C30 alkanes.

In general, the fecal recoveries of n-alkanes were similar between chain lengths in Diets 1 and 2. Therefore, the mean n-alkane fecal output (O_M, mg/d) was plotted against the mean n-alkane intake (I_M, mg/d) to determine the mean fecal recovery (R_2) of all n-alkanes. This method was applied to evaluate the use of one adjusted DMD estimate for each horse, as opposed to separate adjustments for each odd-chain alkane (i.e. C25 to C33). Similarly, the mean n-alkane absorption (E_A, mg/d) was plotted against I_M to estimate endogenous alkane loss. The R_2 was 86.4 ± .52 % (r^2 = .99) which corresponded to an E_A of 15.2 ± .5 % (r^2 = .99) for Diet 1 (Figure 2 and 3, respectively). The R_2 was 84.1 ± 1.7 % (r^2 = .99) which corresponded to an E_A of 15.9 ± 1.7 % (r^2 = .92) for Diet 2 (Figure 4 and 5, respectively).

The D_E [3] and D_{TC} of Diets 1 and 2 are shown in Table 3 and 4, respectively. Comparisons are expressed as the difference in percentage between D_E and D_{TC}. For Diet 1, the D_E for C25, C27, C29, C31, and C33 underestimated D_{TC} by 5.9, 5.3, 4.1, 5.3, and 4.4 %, respectively, but were similar. For Diet 2, the D_E for C25, C27, C29, C31, and C33 underestimated D_{TC} by 17.7, 12.0, 6.1, 9.8, and 11.3 %, respectively ($P < .05$). Underestimates of D_{TC} by the D_E in both diets are a direct result of incomplete recoveries of n-alkanes, with the exception C25 in Diet 1. On the other hand, D_{TC} is subject to a tendency for overestimation of DMD deriving from incomplete collection of feces and orts.

Estimates of DMD using an internal marker are based on the assumption that fecal recovery of the marker is 100 %. Since the fecal recoveries of n-alkanes in this study were incomplete, the D_E for each alkane was adjusted [4] by dividing the concentration of the n-alkane in the feces by the mean recovery of that n-alkane (R_1). For Diet 1, the D_{A1} for C27 to C33 were almost equal to D_{TC} (Table 5). The D_{A1} for C25 underestimated D_{TC} by 6.9 % ($P < .05$), which reinforces the likelihood of C25 fecal recovery (103.7 %) being an error. For Diet 2, the D_{A1} for C29, C31, and C33 were nearly identical to corresponding D_{TC} (Table 6). The D_{A1} for C25 and C27 underestimated D_{TC} by 3.0 and 1.7 %, respectively, but were similar to the D_{TC}.

Estimations of DMD using C35 adjusted for incomplete recovery were more accurate than *in vitro* techniques in lactating sheep grazing perennial ryegrass (Dove et al., 1990). Also, in a study comparing methods for estimating supplement intake and diet digestibility in sheep, estimates of DMD using C33 adjusted for incomplete recovery were almost identical to *in vivo* estimates, and were therefore considered better than estimates obtained from IADF, C31, and lignin (Dove and Coombe, 1992). The results of Exp. 1 also suggest that if the recovery of each n-alkane were to be determined for a specific diet in a digestion balance trial, it may be possible to use those recoveries in a free-range grazing situation to adjust D_E to D_{A1}, thereby improve estimates of DMD.

An alternative method of adjusting the D_E [D_{A2}, equation 5] for Diets 1 and 2 using the mean recovery of all n-alkanes (R_2) is summarized in Table 7. For Diet 1, the D_{A2} was nearly identical to the D_{TC} (54.4 ± 2.1 vs 54.1 ± 1.2, respectively, P = .88). For Diet 2, the mean D_{A2} was also similar to the D_{TC} (61.0 ± .49 vs 60.9 ± 1.33, respectively, P = .90). These results indicate accurate estimates of DMD can be obtained by adjusting for mean recovery of all n-alkanes in forage only diets offered to stalled horses.

Experiment 2

Balance data. The voluntary DMI, FO, and D_{TC} [6] for each horse offered Diets 3 and 4 are shown in Appendix 11. Mean DMI of hay (5.3 ± .16 vs 5.2 ± .20 kg/d, P = .84), concentrate (1.8 ± .08 vs 1.9 ± .04 kg/d, P = .39) and FO (3.0 ± .14 vs 3.1 ± .17 kg/d, P = .89) were similar for horses offered Diet 3 and 4, respectively. The mean D_{TC} was similar for Diet 3 and Diet 4 (57.7 ± 1.2 vs 57.6 ± 1.9 %, respectively, P = .39). Compared to the D_{TC} of the same orchardgrass/alfalfa hay (Diet 2) fed in Exp. 1, the D_{TC} of Diet 4 was lowered when FF was added (60.9 ± 1.3 vs 57.7 ± 1.2, respectively, P = .036), but similar when SS was added (60.9 ± 1.3 vs 57.6 ± 1.9 %, P = .35). In ruminants, DMD was lowered with the addition of concentrates to the diet by possibly adversely affecting the microbes in the rumen, and by lowering pH in the rumen (Minson, 1990). Although a horse is a non-ruminant, the addition of supplements (concentrate) into the diet may cause similar responses in the hind gut. Also, in horses fed hay and hay-plus-concentrate diets, turnover times were 33 and 18 h, respectively (Holland et al., 1998). A shorter retention time caused by the addition of concentrates may partially explain the lower DMD observed in this study.

The partial DMD of FF and SS was calculated using the D_{TC} for each horse consuming orchardgrass/alfalfa hay (Diet 2) in Exp. 1 (Table 4), and individual intakes of hay, concentrate, and the total diet in Exp. 2 (Appendix table 11). Partial dry matter digestibilities of FF and SS were similar (48.5 ± .04 vs 48.0 ± .04 %, respectively, P = .95). The FF concentrate was higher than SS in crude fat (9.6 vs 6.9 %), ADF (26.3 vs 8.7 %), and NDF (38.1 vs 18.0 %) and lower in

NSC (25.8 vs 56.0 %), respectively. A higher NDF and ADF content in roughage diets is negatively correlated with DMD (Minson, 1990); however, the higher NDF and ADF contents in FF did not cause a difference in D_{TC} between Diets 3 and 4 or partial DMD of FF and SS.

Marker data. Concentrations of n-alkanes were higher in orchardgrass/alfalfa hay than in FF and SS concentrates (Appendix table 12). The FF concentrate had higher concentrations of C29 and C31, but similar concentrations of C27, C29 and C33 compared to the SS concentrate. Overall concentrations of n-alkanes in Diets 3 and 4 were calculated using weighted averages for forage and concentrate offered to each horse (Appendix table 13). The most abundant alkanes in Diets 3 and 4 were C31 and C29. The addition of concentrates lowered the concentrations of n-alkanes in both diets.

Fecal concentrations of n-alkanes for consuming Diets 3 and 4 are shown in Appendix table 14. Fecal concentrations of C25 (14.0 ± .49 vs 14.0 ± .31 mg/kg, P = .95), C27 (25.3 ± .78 vs 25.5 ± .67 mg/kg, P = .78), C29 (77.6 ± 2.9 vs 75.9 ± 3.1 mg/kg, P = .36), C31 (157.8 ± 5.3 vs 159.5 ± 8.5 mg/kg, P = .80), and C33 (21.5 ± .62 vs 20.6 ± .67 mg/kg, P = .29) were similar between horses offered Diets 3 and 4, respectively.

Fecal recovery [7] of each odd-chain alkane (R_1) in Diet 3 and 4 are shown in Table 8 and 9, respectively. For Diet 3, the R_1 of C25 was the lowest and C33 was the highest compared to all other n-alkanes (P < .05). The lower recovery of C25 and higher recovery of C33 indicated a trend for increasing recovery with increasing chain length similar to that reported in ruminants (Mayes and Lamb, 1983; Mayes et al., 1986; Dove and Mayes, 1991). However, in ruminants, previously reported ranges of recovery for C25 to C33 were 19.5 to 90.9 % (Mayes et al., 1984) and C27 to C35 were 71.3 to 89.1 % (Mayes et al., 1986a). In our study, the range of recoveries for C25 to C33 was observed to be between 70.7 to 81.2 %. For Diet 4, the R_1 for all n-alkanes were similar, which is in agreement to a previous report of n-alkane recovery in horses (O'Keefe and McMeniman, 1998).

Comparisons of n-alkane recovery between Diets 3 and 4 are shown in Figure 6. Fecal recovery of C25 (70.7 vs 71.4 %, *P* = .95), C27 (73.1 vs 72.3 %, *P* = .92), C29 (75.3 vs 81.8 %, *P* = .078), C31 (76.5 vs 81.3 %, *P* = .19), and C33 (81.2 vs 81.2 %, *P* = .99) did not differ between Diet 3 and 4. The R_1 observed in Diet 3 and 4 are lower than the n-alkane recovery observed for horses consuming orchardgrass/alfalfa hay only (Diet 2) in Exp. 1. The lower fecal recoveries observed in this experiment are most likely due to the addition of concentrates in the diet which lowered total alkane concentrations consumed in Diets 3 and 4. The additional crude fat provided by the FF concentrate in Diet 3 (9.6 %) and the SS concentrate in Diet 4 (6.9 %) may have affected fecal recovery should emulsification be an important factor for absorption of alkanes. However, in cows fed rumenally inert fat in addition to grass hay + concentrate or legume hay + concentrate, recovery of C31 was not affected (Ohajuruka and Palmquist, 1991). In addition, incorporation of palmitic and stearic acids with dosed C28 and C32 did not affect fecal recoveries of the alkanes in sheep fed freshly cut perennial ryegrass (Mayes et al., 1986).

The mean n-alkane fecal output (O_M, mg/d) was plotted against the mean n-alkane intake (I_M, mg/d) to determine the mean fecal recovery (R_2) of all n-alkanes. Also, the mean n-alkane absorption (E_A, mg/d) was plotted against I_M to estimate endogenous alkane loss. The R_2 was 80.9 ± .70 % (r^2 = .99) which corresponded to an E_A of 19.1 ± .71 % (r^2 = .98) for Diet 3 (Figure 7 and 8, respectively). The R_2 was 84.0 ± .69 % (r^2 = .99) which corresponded to an E_A of 15.9 ± .69 % (r^2 = .98) for Diet 4 (Figure 9 and 10, respectively).

The D_E [8] and D_{TC} of Diet 3 and 4 are shown in Table 10 and 11, respectively. For Diet 3, the D_E for C25, C27, C29, C31, and C33 underestimated D_{TC} by 21.3, 17.0, 14.4, 13.5, and 10.7 %, respectively (*P* < .05). For Diet 4, the D_E for C25, C27, C29, C31, and C33 underestimated D_{TC} by 22.23, 16.6, 10.3, 10.8, and 10.2 % (*P* < .05). The underestimation of D_{TC} in both diets using n-alkanes is mainly attributable to their incomplete recovery.

The D_E adjusted [9] for each mean n-alkane recovery (D_{A1}) and D_{TC} of Diet 3 and 4 are shown in Table 12 and 13, respectively. For Diet 3, the D_{A1} for C25, C27, C29, C31, and C33 underestimated D_{TC} by 1.4, .3, .5, .3, and .2 %, respectively, but differences were not significant. For Diet 4, the D_{A1} for C25, C27, C31, and C33 underestimated D_{TC} by 3.1, .3, .7, and .4 %, respectively, and C29 overestimated D_{TC} by .5 %, but were similar to the D_{TC}. These results suggest that if the recovery of n-alkanes were determined for a specific diet in a digestion balance trial, it may be possible to use those recoveries in a free-range grazing situation to adjust D_E.

The D_{A2} [10] for each horse offered Diet 3 and 4 are shown in Table 14. For Diet 3, the D_{A2} tended to underestimate D_{TC} (54.3 ± 1.8 vs 57.7 ± 1.2 %, respectively, P = .21). For Diet 4, the D_{A2} also tended to underestimate D_{TC} (54.6 ± 2.0 vs 57.6 ± 1.9, respectively,P = .19). These data suggest that similar estimates of DMD can be obtained by adjusting for mean recovery of all n-alkanes in forage-plus-concentrate diets offered to stalled horses.

Experiment 3

Balance data. The voluntary DMI, FO and D_{TC} for each stalled horse offered Diet 5 and 6 are shown in Appendix table 15. Mean DMI was similar (10.8 ± .87 vs 9.2 ± .89 kg/d, P = .42) and FO was similar (3.7 ± .23 vs 3.2 ± .34 kg/d, P = .45) for stalled horses offered Diets 5 and 6, respectively. Mean D_{TC} of Diet 5 was similar to Diet 6 (66.1 ± .89 vs 63.5 ± 1.9 %, respectively, P = .85).

Marker data. Concentrations of n-alkanes in Diet 5 did not differ from concentration in Diet 6 (Appendix table 16). Although both diets consisted of a mixture of grass and legume species, the high concentrations of C31 and C29 and low concentrations of C25 are in agreement with data for alkane content in individual grass and legume species (Mayes et al., 1986; Malossini et al., 1990; Dove and Mayes, 1991). Concentrations of n-alkanes in individual diet components are reported in Appendix table 17. The n-alkane concentrations in alfalfa observed in this experiment were consistently lower than previously reported values (Malossini et al., 1990). In addition, the concentrations of C27 and C29 were lower and concentrations of C31 and

C33 higher for our white clover compared to other reports of n-alkane concentrations in white clover (Malossini et al., 1990). Lower concentration observed in our study may be attributed to differences in climate, plant parts sampled, and plant maturity (Dove et al., 1992). The higher concentrations of n-alkanes observed in the alfalfa and white clover compared to the tall fescue and bluegrass is in agreement with previous studies that report higher concentrations of n-alkanes in legumes compared to grass species (Malossini et al., 1990; Dove et al., 1995). This is the first study to report concentrations of odd-chain alkanes (C25 to C33) in tall fescue and bluegrass species.

Fecal concentrations of n-alkanes for stalled and pastured horses offered Diet 5 and 6 are shown in Appendix table 18 and 19, respectively. For Diet 5, fecal concentrations of C33 were similar (87.0 ± 3.4 vs 88.3 ± 10.8 mg/kg, P = .93), whereas fecal concentrations of C25 (9.1 ± 1.1 vs 84.2 ± 16.7 mg/kg, P = .018), C27 (19.4 ± 4.1 vs 114.8 ± 28.4 mg/kg, P = .044), C29 (97.6 ± 14.9 vs 173.4 ± 28.4 mg/kg, P = .044), and C31 (275.0 ± 11.8 vs 391.0 ± 10.3 mg/kg, P = .009) were higher for horses remaining on pasture compared to stall-fed horses. For Diet 6, fecal concentrations of C25 (10.1 ± 1.8 vs 68.9 ± 8.3 mg/kg, P = .003) and C27 (20.9 ± 5.5 vs 72.6 ± 5.9 mg/kg, P = .001) were higher for horses remaining on pasture compared to stall-fed horses. However, fecal concentrations were similar for C29 (138.9 ± 27.8 vs 181.2 ± 37.7 mg/kg, P = .40), C31 (338.5 ± 41.0 vs 365.7 ± 38.6 mg/kg, P = .66), and C33 (118.8 ± 3.3 vs 117.4 ± 4.7 mg/kg, P = .85). The higher fecal concentrations of alkanes in the feces of horses on pasture suggests a higher n-alkane intake by those horses, perhaps by selectively grazing the legumes, which had higher concentrations of n-alkanes compared to the grasses.

The R_1 for stalled horses offered Diet 5 and 6 are shown in Table 15 and 16, respectively. For Diet 5, the R_1 of all n-alkanes were similar. For Diet 6, C25 and C27 had the highest recovery and were similar. Recoveries of C27, C29, C31, and C33 were also similar. The R_1 for both Diets 5 and 6 were consistently lower than recoveries observed for Diets 1 and 2 in Exp. 1, and Diets 3 and 4 in Exp. 2. These data suggest that horses are capable of metabolizing the

longer odd-chain alkanes (C29, C31, and C33) in freshly cut pasture to a greater extent then mixed grass/legume hay and hay-plus-concentrate diets offered in Exp. 1 and 2, respectively. This may be due to a different microbial population in the hindgut of horse fed pasture compared to when they consumed hay and hay-plus-concentrate diets. Also, the pastures were at an earlier stage of maturity compared to the hays in Exp. 1 and 2, which may have influenced the availability of n-alkanes to metabolism by gut microflora.

Comparison of R_1 for stalled horses consuming Diets 5 and 6 is shown in Figure 11. Fecal recoveries of n-alkanes were similar between stalled horses offered Diets 5 and 6. Fecal recoveries of C29, C31, and C33 in both diets were lower than recoveries reported in ruminants (Mayes and Lamb, 1983; Mayes et al., 1986; Dove and Mayes, 1991) and horses (O'Keefe and McMeniman, 1998). In this experiment, the intake level of horses maintained in stalls was probably lower than the horses remaining on pasture. The difference in recovery between stall-fed and pastured horses may be due to a longer retention time in the hindgut of stall-fed horses, which would increase time for metabolism of n-alkanes.

The mean n-alkane fecal output (O_M, mg/d) was plotted against the mean n-alkane intake (I_M, mg/d) to determine the mean fecal recovery (R_2) of all n-alkanes. Also, the mean n-alkane absorption (E_A, mg/d) was plotted against I_M to estimate endogenous alkane loss. The R_2 was 61.3 ± .53 % (r^2 = .99) which corresponded to an E_A of 38.7 ± .53 % (r^2 = .99) for Diet 5 (Figure 12 and 13, respectively). For Diet 6, the R_2 was 63.6 ± .31 % (r^2 = .99), corresponding to an E_A of 36.4 ± .31 % (r^2 = .99) for Diets 6 (Figure 14 and 15, respectively).

The D_E and D_{TC} for stalled horses offered Diet 5 and 6 are shown in Table 17 and 18, respectively. For stalled horses offered Diet 5, the D_E for C25 and C33 underestimated D_{TC} by 11.8 and 14.7 %, respectively, but were similar to the D_{TC}. The D_E for C27, C29, and C31 underestimated D_{TC} by 14.8, 16.5, and 17.1 % ($P < .05$). For stalled horses offered Diet 6, the D_E for C25 underestimated D_{TC} by 7.4 %, but was similar to the D_{TC}. The D_E for C27, C29, C31,

and C33 underestimated D_{TC} by 10.4, 20.0, 20.0, and 20.4 % ($P < .05$). Underestimations of the DTC by DE were expected based on the incomplete recovery of n-alkanes.

The D_E and D_{TC} for pastured horses offered Diet 5 and 6 are shown in Table 19 and 20 respectively. For Diet 5, the D_E for C29 and C31 underestimated D_{TC} by 5.9 and 1.9 %, respectively, but was similar to D_{TC}, whereas the D_E for C33 underestimated D_{TC} by 13.3 % ($P < .05$). The D_E for C25 and C27 overestimated D_{TC} by 28.8 and 24.4 %, respectively ($P < .05$). For Diet 6, the D_E for C29 underestimated D_{TC} by 7.0 %, but was similar to D_{TC}. The D_E estimates of C25 and C27 overestimated D_{TC} by 29.7 and 22.5 %, respectively ($P < .05$). The high D_E obtained for C25 and C27 most likely attributed to the high concentrations of C25 and C27 in the feces compared to concentration of n-alkanes in the pasture sampled. Since these horses were maintained on pasture, they could have selectively grazed pasture that was unrepresentative of the pasture sampled and used for the estimation. Also, mineral oil was used as lubrication for the rectal grab samples on the pastured horses. Mineral oil may contain sufficient amounts of the lower odd-chain alkanes, which would have apparently increased the fecal concentration of C25 and C27 in the feces. As this was not perceived until the completion of the experiment, mineral oil was not analyzed for alkane content.

The D_E adjusted (D_{A1}) using the mean of each n-alkane (R_1) and D_{TC} for stalled and pastured horses offered Diet 5 in Table 21. For stalled horses offered Diet 5, the D_{A1} for C25 and C27 underestimated D_{TC} by .24 and .4 %, respectively, and were similar to the D_{TC}. The D_{A1} for C29, C31, and C33 overestimated D_{TC} by 2.7, 1.5, and 3.4 %, respectively, but were similar to the D_{TC}. For pastured horses offered Diet 5, the D_{A1} for C33 overestimated D_{TC} by 2.1 %, but was similar to the D_{TC}. All other n-alkane based estimations of D_{A1} overestimated D_{TC} between 11.1 and 30.0 % and were different than the D_{TC} estimate ($P < .05$).

The D_{A1} and D_{TC} estimates for stalled and pastured horses offered Diet 6 are shown in Table 22. For stalled horses offered Diet 6, the D_{A1} for C27 underestimated D_{TC} by .7 %, but was similar to the D_{TC}. The D_{A1} for C25, C29, C31, and C33 overestimated D_{TC} by .3, .1, .4, and

.2 %, respectively, but was similar to the D_{TC}. For pastured horses offered Diet 6, the D_{A1} for C29 and C31 overestimated D_{TC} by 8.4 and 3.3 %, respectively, and the D_{A1} for C33 underestimated D_{TC} by .23 %, but were not different from the D_{TC}. The D_{A1} for C25 and C27 overestimated D_{TC} by 31.0 and 26.1 %, respectively ($P < .05$).

Adjusting the D_E for mean recovery of each n-alkane for stalled horses in both Diets 5 and 6 provided accurate estimations when compared to the D_{TC}. For pastured horses, adjusting the D_E using the mean recovery of each n-alkane obtained for stalled horses increased the D_E of Diets 5 and 6. For Diet 5, this allowed only the C33 alkane estimate to be similar to D_{TC}, whereas for Diet 6, C31, and C33 alkane estimates were improved by adjusting for incomplete recovery.

The D_E adjusted (D_{A2}) using the mean recovery of all n-alkanes and D_{TC} for stalled and pastured horses offered Diet 5 and 6 are shown in Table 23. For stalled horses offered Diet 5, the D_{A2} was similar to the D_{TC} (69.4 ± 1.0 vs 66.1 ± .89 %, $P = .053$). For pastured horses offered Diet 5, the D_{A2} overestimated the D_{TC} (82.4 ± .97 vs 66.1 ± .89 %, respectively, $P = .0003$). For stalled horses offered Diet 6, the D_{A2} was similar to the D_{TC} (64.2 ± 2.5 vs 63.9 ± 2.3 %, respectively, $P = .89$). For pastured horses offered Diet 6, the D_{A2} overestimated and was different from the D_{TC} (72.6 ± .87 vs 63.9 ± 2.3 %, respectively, $P = .01$). These results indicate that adjusting the D_E for recovery of all n-alkanes leads to inaccuracies when compared the D_{TC} in pasture conditions.

During the course of this study, a separate investigator conducted an adjacent study; dosing chromium three times daily to stalled and pastured horses to evaluate its use as an external marker to estimate FO. Estimates of FO using chromium and DMD using alkanes can be used to estimate DMI for the pastured horses. Therefore, a more thorough interpretation of these data will be possible when FO data becomes available from the adjacent study.

General Discussion. Determination of fecal recoveries of odd-chain alkanes (C25 to C33) in Exp. 1, 2, and 3 revealed less than 100 % recovery, with one exception, and recovery was similar for chain lengths. These data suggest horses are capable of some digestion of n-alkanes. Thus, underestimates of D_{TC} were obtained when compared to D_E using odd-chain alkanes in diets of stall-fed horses. For pastured horses in Exp. 3, D_E were less consistent, resulting in both overestimation and underestimation of D_{TC}. Two methods of adjusting the D_E for alkane recovery were established. In nearly all instances, adjusting the D_E using R_1 provided almost identical estimates compared to D_{TC} of diets fed to stalled horses in Exp. 1, 2, and 3. Adjusting the D_E using R_1 for pastured horses in Exp. 3 resulted in less consistent estimates of DMD compared to the D_{TC} for both diets. Alternatively, when D_E was adjusted using R_2, the mean D_{A2} for stalled horses was similar to the D_{TC} of their respective diets. However, the mean D_{A2} was not similar to D_{TC} for pastured horses offered Diets 5 and 6 in Exp. 3. These results indicate that adjusting the D_E using R_1 provided more accurate estimations of DMD compared to adjusting D_E using R_2 in pasture conditions.

Implications

Fecal recoveries of odd-chain alkanes (C25 to C33) in horses fed forage and forage-plus-concentrate diets were less than 100 %, and did not appear to increase with increasing chain length. Since recoveries of odd-chain alkanes were similar between chain length, their use as internal markers in conjunction with an even-chain alkane as an external marker may accurately estimate pasture DMI by horses. Accurate estimates of DMD can be obtained by adjusting for mean recovery of the odd-chain alkane marker in stall-fed horses. Under pasture conditions, adjusting estimates of DMD using the longer odd-chain alkanes (i.e. C31 and C33) may provide better estimates of DMD than shorter odd-chain alkanes.

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Tables

Table 1. Fecal recovery (R_1, %) of each odd-chain alkane for horses offered tall fescue/alfalfa hay (Diet 1) in Experiment 1[a]

	Diet 1				
	n – alkanes				
Horse	C25	C27	C29	C31	C33
1	137.05	114.36	103.26	97.72	111.34
2	91.70	73.35	78.49	80.23	74.78
3	110.58	106.99	108.91	102.86	104.83
4	90.95	72.95	77.05	79.76	81.63
5	90.75	74.34	80.18	81.87	79.11
6	110.03	106.22	97.10	97.74	101.28
7	116.42	107.02	99.35	88.61	96.39
8	81.90	63.08	67.40	72.58	72.55
Mean ± SE[b]	103.67 ± 6.42[c]	89.79 ± 7.29[d]	88.97 ± 5.29[d]	87.67 ± 3.81[d]	90.24 ± 5.29[d]

[a] Mean of Periods 1 and 2
[b] Standard error of the mean (n = 8)
[c,d] Means within row with different superscript differ ($P < .05$)

Table 2. Fecal recovery (R_1, %) of each odd-chain alkane for horses offered orchardgrass/alfalfa hay (Diet 2) in Experiment 1[a]

	Diet 2				
	n-alkanes				
Horse	C25	C27	C29	C31	C33
1	71.97	84.29	98.15	87.13	91.39
2	76.64	72.31	78.18	71.16	70.43
3	67.38	75.03	86.91	78.48	79.57
4	81.10	77.07	91.14	80.43	76.82
5	89.38	91.74	107.51	96.16	81.94
6	59.35	70.28	85.02	78.06	76.04
7	71.12	82.10	88.38	76.47	79.90
8	83.74	83.41	97.96	91.35	81.53
Mean ± SE[b]	75.08 ± 3.41[c]	79.53 ± 2.53[c,d]	91.66 ± 3.25[e]	82.40 ± 2.96[d]	79.70 ± 2.12[c,d]

[a] Mean of Periods 1 and 2
[b] Standard error of the mean (n = 8)
[c,d,e] Means within row with different superscript differ ($P < .05$)

Table 3. Total collection DMD (D_{TC}, %) and estimated DMD (D_E, %) for each n-alkane for horses offered tall fescue/alfalfa hay (Diet 1) in Experiment 1

	Diet 1					
		D_E				
Horse	D_{TC}	C25	C27	C29	C31	C33
1	49.38	63.06	55.74	50.98	48.20	54.54
2	57.49	39.75	42.05	45.84	47.02	43.16
3	53.49	57.94	56.53	57.30	54.79	55.64
4	55.43	36.31	38.90	42.15	44.12	45.40
5	50.83	29.58	33.85	38.67	39.94	37.84
6	51.39	55.82	54.23	49.93	50.26	52.00
7	57.36	63.37	60.15	57.08	51.87	55.76
8	57.15	39.57	48.97	57.82	54.06	52.84
Mean ± SE[a]	54.07 ± 1.15[b]	48.18 ± 4.70[b]	48.80 ± 3.36[b]	49.97 ± 2.58[b]	48.78 ± 1.79[b]	49.65 ± 2.36[b]

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

[b] Means within row with like superscripts do not differ ($P > .05$)

Table 4. Total collection DMD (D_{TC}, %) and estimated DMD (D_E, %) for each n-alkane for horses offered orchardgrass/alfalfa hay (Diet 2) in Experiment 1

	Diet 2					
		D_E				
Horse	D_{TC}	C25	C27	C29	C31	C33
1	59.67	43.97	52.16	58.91	53.72	55.87
2	66.35	49.57	53.47	56.96	52.72	52.23
3	58.69	43.11	48.90	55.89	51.15	51.82
4	60.25	41.95	46.82	55.03	49.04	46.64
5	55.45	42.75	51.44	58.56	53.67	45.63
6	64.14	49.32	56.10	59.22	52.87	54.89
7	63.95	32.00	32.07	36.43	40.96	40.94
8	56.98	40.99	48.42	56.08	52.90	47.23
Mean ± SE[a]	60.69 ± 1.34[b]	42.96 ± 1.94[e]	48.67 ± 2.59[d]	54.64 ± 2.66[c]	50.88 ± 1.52[c,d]	49.41 ± 1.81[c,d]

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

[b,c,d] Means within row with different superscripts differ ($P < .05$)

Table 5. Total collection DMD (D_{TC}, %) and estimates of DMD adjusted (D_{A1}, %) using the mean recovery of each n-alkane (R_1) for horses offered tall fescue/alfalfa hay (Diet 1) in Experiment 1

	Diet 1					
		D_{A1}				
Horse	D_{TC}	C25	C27	C29	C31	C33
1	49.36	56.22	51.91	49.92	49.89	52.96
2	57.49	46.49	58.90	58.96	58.35	56.22
3	53.47	50.16	52.77	56.38	56.26	54.10
4	55.44	43.42	56.66	56.16	56.07	57.95
5	50.81	37.45	53.08	53.53	52.79	52.13
6	51.34	47.63	52.00	48.85	51.89	50.34
7	57.37	56.58	60.20	56.15	53.44	54.23
8	57.13	39.60	51.82	55.33	53.59	54.51
Mean ± SE[a]	54.05 ± 1.15[b]	47.20 ± 2.49[c]	54.67 ± 1.21[b]	54.41 ± 1.22[b]	54.04 ± .96[b]	54.05 ± .83[b]

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

[b,c] Means within row with different superscripts differ ($P < .05$)

Table 6. Total collection DMD (D_{TC}, %) and estimates of DMD adjusted (D_{A1}, %) using the mean recovery for each n-alkane (R_1) for horses offered orchardgrass/alfalfa hay (Diet 2) in Experiment 1

	Diet 2					
		D_{A1}				
Horse	D_{TC}	C25	C27	C29	C31	C33
1	59.69	62.20	62.72	63.18	62.96	63.94
2	66.30	58.29	62.25	59.68	59.92	62.89
3	61.60	61.62	60.18	60.47	60.90	60.62
4	59.02	51.98	56.85	57.86	56.80	58.55
5	55.49	52.65	53.55	61.18	60.73	57.77
6	64.10	59.24	60.24	62.20	63.23	61.46
7	63.92	65.81	65.79	63.45	62.28	63.13
8	56.94	51.19	52.12	58.85	60.07	59.01
Mean ± SE[a]	60.88 ± 1.33[d]	57.87 ± 1.91[d]	59.21 ± 1.66[d]	60.85 ± .71[d]	60.86 ± .73[d]	60.92 ± .82[d]

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

[d] Means within row with like superscript do not differ ($P > .05$)

Table 7. Total collection DMD (D_{TC}, %) and estimates of DMD adjusted (D_{A2}, %) using the mean recovery of all n-alkanes (R_2) for horses offered tall fescue/alfalfa hay (Diet 1) and orchardgrass/alfalfa hay (Diet 2) in Experiment 1

	Diet 1		Diet 2	
Horse	D_{TC}	D_{A2}	D_{TC}	D_{A2}
1	49.36	57.48	59.69	62.02
2	57.49	53.06	66.30	61.08
3	53.47	61.74	61.60	59.67
4	55.44	50.74	59.02	58.00
5	50.81	46.86	55.49	62.29
6	51.34	57.56	64.10	61.50
7	57.37	60.86	63.92	62.02
8	57.13	47.13	56.94	60.18
Mean ± SE[a]	54.05[b] ± 1.15	54.43[b] ± 2.07	60.88[c] ± 1.33	61.00[c] ± .49

[a] Standard error of the mean (n = 8)
[b] Means within row with similar superscripts do not differ (P = .88)
[c] Means within row with similar superscripts do not differ (P = .91)

Table 8. Fecal recovery (R_1, %) of each odd-chain alkane for horses offered orchardgrass/alfalfa hay and FF concentrate (Diet 3) in Experiment 2

	Diet 3				
	n-alkanes				
Horse	C25	C27	C29	C31	C33
1	87.40	90.89	91.80	94.38	101.97
2	60.41	65.40	73.92	76.37	72.39
3	90.51	89.16	80.78	79.22	92.53
4	50.51	54.01	58.53	59.54	59.93
5	64.10	70.14	83.33	84.94	81.25
6	59.23	62.74	64.76	68.82	75.12
7	93.96	90.45	81.22	80.26	94.77
8	59.25	61.95	68.37	68.32	71.41
Mean ± SE[a]	70.67 ± 6.02[b]	73.09 ± 5.24[b,c]	75.34 ± 3.88[b,c]	76.48 ± 3.84[b,c]	81.17 ± 5.01[c]

[a] Standard error of the mean (n = 8)
[b,c] Means within row with different superscripts differ (P < .05)

Table 9. Fecal recovery (R_1, %) of each odd-chain alkane for horses offered orchardgrass/ alfalfa hay and SS concentrate (Diet 4) in Experiment 2

	Diet 4				
	n-alkanes				
Horse	C25	C27	C29	C31	C33
1	67.31	80.59	106.02	106.89	95.80
2	68.93	66.44	73.86	72.03	69.95
3	63.21	72.49	90.69	91.26	84.48
4	85.84	77.66	75.62	74.95	87.21
5	93.77	84.04	83.83	81.40	83.46
6	48.31	56.42	71.75	75.15	71.09
7	49.15	55.48	71.87	70.82	64.71
8	94.61	85.55	80.48	77.46	93.22
Mean ± SE[a]	71.39 ± 6.50[b]	72.33 ± 4.18[b]	81.7 ± 4.17[b]	81.25 ± 4.31[b]	81.24 ± 4.03[b]

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

[b] Means within row with like superscript do not differ ($P > .05$)

Table 10. Total collection DMD (D_{TC}, %) and estimated DMD (D_E, %) for each n-alkane for horses offered orchardgrass/ alfalfa hay and FF (Diet 3) in Experiment 2

	Diet 3					
		D_E				
Horse	D_{TC}	C25	C27	C29	C31	C33
1	55.40	49.52	51.47	51.95	53.30	56.75
2	61.04	32.72	40.43	47.29	48.99	46.18
3	55.16	50.48	49.74	44.52	43.44	51.57
4	60.44	18.37	26.70	32.34	33.45	33.93
5	52.60	22.65	32.43	43.13	44.22	41.67
6	61.86	36.24	39.98	41.81	45.70	49.93
7	55.77	52.77	50.88	45.31	44.48	53.09
8	58.84	27.62	33.57	39.81	39.77	42.37
Mean ± SE[a]	57.64 ± 1.19[b]	36.30 ± 4.71[c]	40.65 ± 3.32[c,d]	43.27 ± 2.03[c,d]	44.17 ± 2.09[c,d]	46.94 ± 2.61[d]

[a] Standard error of the mean (n = 8)
[b,c,d] Means within row with different superscripts differ ($P < .05$)

Table 11. Total collection DMD (D_{TC}, %) and estimated DMD (D_E, %) for each n-alkane for horses offered orchardgrass/ alfalfa hay and SS (Diet 4) in Experiment 2

	Diet 4					
		D_E				
Horse	D_{TC}	C25	C27	C29	C31	C33
1	54.85	32.92	43.97	57.41	57.76	52.87
2	60.37	35.39	39.35	43.72	41.81	41.45
3	59.98	36.70	44.80	55.88	56.16	52.64
4	54.81	40.45	41.80	40.22	39.68	48.17
5	52.72	43.49	43.75	43.60	41.92	43.36
6	64.39	26.91	37.32	50.51	52.68	50.13
7	64.07	27.51	35.68	50.15	49.34	44.71
8	49.36	39.48	40.81	37.07	34.62	45.68
Mean ± SE[a]	57.57 ± 1.93[b]	35.35 ± 2.11[c]	40.94 ± 1.17[c,d]	47.32 ± 2.59[d]	46.75 ± 2.98[d]	47.37 ± 1.51[d]

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

[b,c,d] Means within row with different superscripts differ ($P < .05$)

Table 12. Total collection DMD (D_{TC}, %) and estimated DMD adjusted (D_{A1}, %) using the mean recovery of each n-alkane (R_1) for horses offered orchardgrass/alfalfa hay and FF (Diet 3) in Experiment 2

	Diet 3					
		D_{A1}				
Horse	D_{TC}	C25	C27	C29	C31	C33
1	55.42	58.21	59.57	61.73	62.33	60.60
2	61.11	60.60	62.54	62.56	63.12	61.66
3	55.24	59.01	58.13	55.82	54.37	55.88
4	60.39	52.19	53.91	51.94	51.89	52.93
5	52.55	54.70	57.51	59.60	59.68	58.44
6	61.83	47.22	49.99	53.66	56.19	54.39
7	55.82	60.90	59.08	56.45	55.21	57.27
8	58.88	57.61	58.23	55.72	56.46	58.94
Mean ± SE[a]	57.66 ± 1.18[b]	56.31 ± 1.66[b]	57.37 ± 1.35[b]	57.18 ± 1.34[b]	57.41 ± 1.39[b]	57.51 ± 1.06[b]

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

[b] Means within row with like superscript do not differ ($P > .05$)

Table 13. Total collection DMD (D_{TC}, %) and estimated DMD adjusted (D_{A1}, %) using the mean recovery of each n-alkane (R_1) for horses offered orchardgrass/alfalfa hay and SS (Diet 4) in Experiment 2

	Diet 4					
		D_A				
Horse	D_{TC}	C25	C27	C29	C31	C33
1	54.77	61.77	62.88	63.77	63.66	62.76
2	60.38	44.57	52.43	55.85	55.51	51.13
3	60.03	63.92	63.43	62.46	62.29	62.58
4	54.81	48.92	54.36	53.11	53.88	56.74
5	52.69	51.52	55.89	55.76	55.59	52.73
6	64.47	58.34	58.48	57.90	59.29	60.59
7	64.01	58.68	57.39	64.59	56.41	56.31
8	49.33	48.08	53.58	50.63	50.01	54.66
Mean ± SE[a]	57.56 ± 1.94[b]	54.47 ± 2.51[b]	57.31 ± 1.45[b]	58.01 ± 1.82[b]	57.08 ± 1.58[b]	57.19 ± 1.56[b]

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

[b] Means within row with like superscript do not differ ($P > .05$)

Table 14. Total collection DMD (D_{TC}, %) and estimates of DMD adjusted (D_{A2}, %) using the mean recovery of all n-alkanes (R_2) 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	D_{TC}	D_{A2}	D_{TC}	D_{A2}
1	55.42	61.88	54.77	62.38
2	61.11	57	60.38	51.11
3	55.24	55.7	60.03	61.37
4	60.39	44.86	54.81	50.2
5	52.55	52.9	52.69	51.87
6	61.83	54.84	64.47	57.66
7	55.82	56.59	64.01	55.44
8	58.88	50.49	49.33	47.07
Mean ± SE[a]	57.66 ± 1.18 [b]	54.28 ± 1.78 [b]	57.56 ± 1.94 [c]	54.64 ± 2.0 [c]

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

[b] Means within row with similar superscripts do not differ (P = .88)

[c] Means within row with similar superscripts do not differ (P = .90)

Table 15. Fecal recovery (R_1, %) of each odd-chain alkane for stalled horses offered tall fescue/alfalfa pasture (Diet 5) in Experiment 3

	Diet 5				
	n-alkanes				
Horse	C25	C27	C29	C31	C33
1	89.19	73.33	65.51	70.18	71.63
2	57.68	62.90	64.26	62.82	71.48
3	63.47	71.99	74.53	72.49	81.64
4	105.21	74.33	44.48	49.09	38.87
Mean ± SE[a]	78.89 ± 11.13[b]	70.64 ± 2.62[b]	62.20 ± 6.33[b]	63.65 ± 5.27[b]	65.91 ± 9.32[b]

[a] Standard error of the mean (n = 4)
[b] Means within row with like superscript do not differ ($P > .05$)

Table 16. Fecal recovery (R_1, %) of each odd-chain alkane for stalled horses offered bluegrass/white clover pasture (Diet 6) in Experiment 3

	Diet 6				
	n-alkanes				
Horse	C25	C27	C29	C31	C33
1	72.05	69.87	58.33	59.79	60.60
2	58.67	56.47	40.75	41.02	38.44
3	120.75	101.28	88.63	82.35	80.29
4	78.42	76.96	70.09	74.07	79.61
Mean ± SE[a]	82.47 ± 13.41[b]	76.15 ± 9.39[b,c]	64.45 ± 10.06[c]	64.31 ± 9.05[c]	64.73 ± 9.88[c]

[a] Standard error of the mean (n = 4)
[b,c] Means within row with different superscripts differ ($P < .05$)

Table 17. Total collection DMD (D_{TC}, %) and estimated DMD (D_E, %) for each n-alkane for stalled horses offered tall fescue/alfalfa pasture (Diet 5) in Experiment 3

	Diet 5					
		D_E				
Horse	D_{TC}	C25	C27	C29	C31	C33
1	64.04	59.69	50.97	45.11	48.77	49.80
2	65.64	40.42	45.37	46.53	45.30	51.93
3	66.26	46.84	53.13	54.73	53.45	58.67
4	68.39	69.96	55.77	52.13	48.57	53.16
Mean ± SE[a]	66.07 ± .89[b]	54.23 ± 6.60[b,c]	51.31 ± 2.21[c]	49.62 ± 2.28[c]	49.02 ± 1.68[c]	53.39 ± 1.89[b,c]

[a] Standard error of the mean (n = 4)
[b,c] Means within row with different superscripts differ ($P < .05$)

Table 18. Total collection DMD (D_{TC}, %) and estimated DMD (D_E, %) for each n-alkane for stalled horses offered bluegrass/white clover pasture (Diet 6) in Experiment 3

	Diet 6					
		D_E				
Horse	D_{TC}	C25	C27	C29	C31	C33
1	70.79	59.45	58.19	49.92	51.14	51.79
2	60.83	51.77	50.85	46.04	48.94	52.49
3	61.41	54.38	52.60	34.32	34.75	30.38
4	62.18	58.51	50.53	43.47	39.16	37.60
Mean ± SE[a]	63.47 ± 1.85[b]	56.03 ± 1.80[b,c]	53.04 ± 1.77[c,d]	43.44 ± 3.31[d]	43.49 ± 3.91[d]	43.06 ± 5.45[d]

[a] Standard error of the mean (n = 4)
[b,c,d] Means within row with different superscripts differ ($P < .05$)

Table 19. Mean total collection DMD (D_{TC}, %) from stalled horses and estimated DMD (D_E, %) for each n-alkane for pastured horses offered tall fescue/alfalfa pasture (Diet 5) in Experiment 3

		Diet 5				
		D_E				
Horse	D_{TC}	C25	C27	C29	C31	C33
5	-	96.55	95.90	73.84	67.74	58.75
6	-	94.27	85.66	71.28	61.46	46.10
7	-	94.77	92.53	72.35	63.50	46.81
8	-	93.96	87.74	70.34	64.02	59.52
Mean ± SE[a]	66.07 ± .89[b,c]	94.88 ± .58[d]	90.46 ± 2.31[d]	71.95 ± .75[b]	64.18 ± 1.31[c]	52.80 ± 3.67[e]

[a] Standard error of the mean (n = 4)
[b,c,d,e] Means within row with different superscripts differ ($P < .05$)

Table 20. Mean total collection DMD (D_{TC}, %) from stalled horses and estimated DMD (D_E, %) for each n-alkane for pastured horses offered bluegrass/white clover pasture (Diet 6) in Experiment 3

		Diet 6				
		D_E				
Horse	D_{TC}	C25	C27	C29	C31	C33
5	-	95.84	93.64	53.73	42.80	34.77
6	-	93.42	81.25	63.01	53.36	44.30
7	-	88.13	77.09	57.69	52.19	51.57
8	-	95.15	91.97	51.30	44.20	40.51
Mean ± SE[a]	63.47 ± 1.85[b]	93.14 ± 1.74[c]	85.99 ± 4.04[c]	56.43 ± 2.56[b,d]	48.14 ± 2.70[d]	42.79 ± 3.52[d]

[a] Standard error of the mean (n = 4)
[b,c,d] Means within row with different superscripts differ ($P < .05$)

Table 21. Total collection DMD (D_{TC}, %) and estimates of DMD adjusted (D_{A1}, %) using the mean recovery of each n-alkane (R1) for horses offered tall fescue/alfalfa pasture (Diet 5) in Experiment 3

	Diet 5					
		D_{A1}				
Horse	D_{TC}	C25	C27	C29	C31	C33
Stalled						
1	64.04	60.82	63.80	69.82	69.45	72.27
2	65.61	63.91	63.16	62.89	62.99	63.20
3	66.28	67.80	68.39	68.58	68.51	68.36
4	68.35	70.80	67.34	73.67	69.33	74.12
Mean ± SE[a]	66.07 ± .89[b]	65.83 ± 2.19[b]	65.67 ± 1.29[b]	68.74 ± 2.23[b]	67.57 ± 1.54[b]	69.48 ± 2.42[b]
Pasture						
5	-	94.12	90.95	83.69	78.54	77.64
6	-	96.64	96.97	85.62	80.76	77.21
7	-	96.53	90.33	80.07	73.92	58.73
8	-	96.83	94.96	80.81	75.31	59.28
Mean ± SE	66.07 ± .89[b]	96.03 ± .55[e]	93.3 ± 1.38[e]	82.55 ± 1.11[d]	77.13 ± 1.34[c,d]	68.21 ± 4.61[b,c]

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

[b,c,d,e] Means within row with different superscripts differ ($P < .05$)

Table 22. Total collection DMD (D_{TC}, %) and estimates of DMD adjusted (D_{A1}, %) using the mean recovery of each n-alkane (R1) for horses offered bluegrass/white clover pasture (Diet 6) in Experiment 3

	Diet 6					
		D_{A1}				
Horse	D_{TC}	C25	C27	C29	C31	C33
Stalled						
1	70.82	69.49	69.30	67.84	67.29	66.20
2	61.18	59.07	62.61	57.51	59.75	58.67
3	61.41	62.78	60.98	63.43	62.47	62.95
4	62.18	63.71	63.92	65.35	65.82	66.69
Mean ± SE[a]	63.47 ± 1.85[b]	63.76 ± 2.16[b]	64.20 ± 1.80[b]	63.53 ± 2.20[b]	63.84 ± 1.69[b]	63.63 ± 1.85[b]
Pasture						
5	-	95.05	86.24	76.25	68.78	60.95
6	-	91.07	83.18	72.83	68.00	66.05
7	-	95.65	93.67	68.50	65.58	64.68
8	-	96.27	94.99	70.07	64.72	61.27
Mean ± SE	63.47 ± 1.85[b]	94.51 ± 1.17[c]	89.52 ± 2.86[c]	71.91 ± 1.70[b]	66.77 ± .96[b]	63.24 ± 1.26[b]

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

[b,c] Means within row with different superscripts differ ($P < .05$)

Table 23. Total collection digestibility (D_{TC}, %) and estimates of DMD adjusted (D_{A2}, %) using the mean recovery of all n-alkanes (R_2) for horses offered tall fescue/alfalfa pasture (Diet 5) and bluegrass/white clover pasture (Diet 6) in Experiment 3

	Diet 5		Diet 6	
Horse	D_{TC}	D_{A2}	D_{TC}	D_{A2}
Stalled				
1	64.04	68.5	70.82	69.08
2	65.61	67.23	61.18	67.53
3	66.28	72.06	61.41	58.27
4	68.35	69.8	62.18	61.93
Mean ± SE[a]	66.07 ± .89 [b]	69.40 ± 1.0 [b]	63.90 ± 2.32 [c]	64.20 ± 2.50 [c]
Pasture				
1	64.04	80.31	70.82	75.03
2	65.61	84.42	61.18	72.76
3	66.28	81.19	61.41	71.03
4	68.35	83.6	62.18	71.71
Mean ± SE	66.07 ± .89 [d]	82.38 ± 1.78 [d]	63.90 ± 2.32 [e]	72.63 ± .87 [f]

[a] Standard error of the mean (n = 4)
[b] Means within row with similar superscripts do not differ (P = .053)
[c] Means within row with different superscripts differ (P = .89)
[d] Means within row with similar superscripts do not differ (P = .0003)
[e,f] Means within row with different superscripts differ (P = .01)

Figures

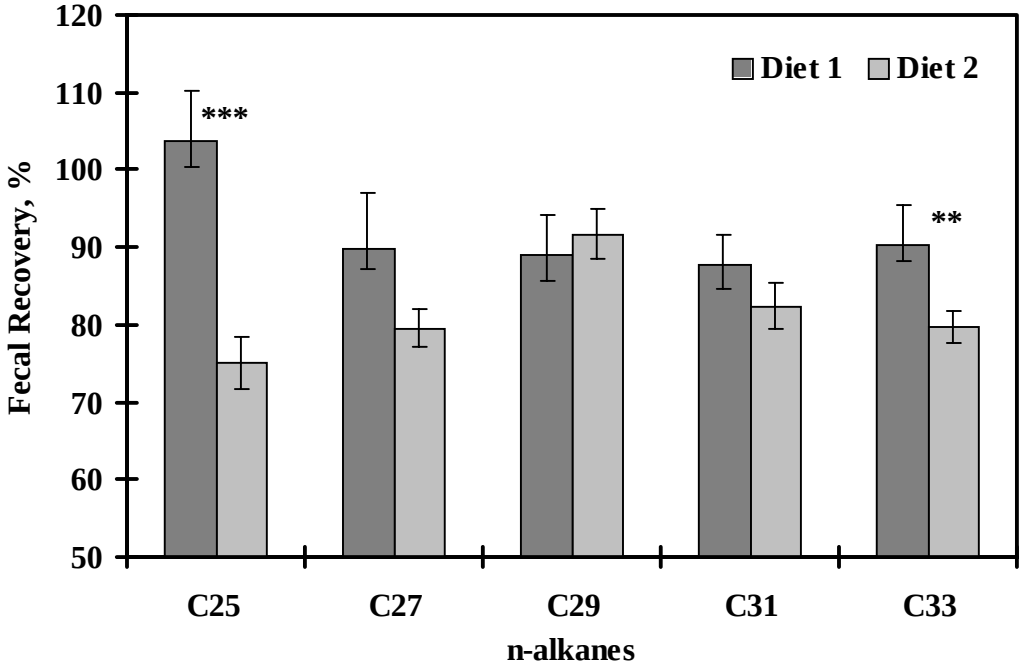

Figure 1. Fecal recovery (mean[a] ± SE[b]) of n-alkanes for horses receiving tall fescue/alfalfa hay (Diet 1) and orchardgrass/alfalfa hay (Diet 2) in Experiment 1. [a] Mean of Periods 1 and 2; [b] Standard error of the mean (n = 8); *** ($P < .001$); ** ($P < .10$)

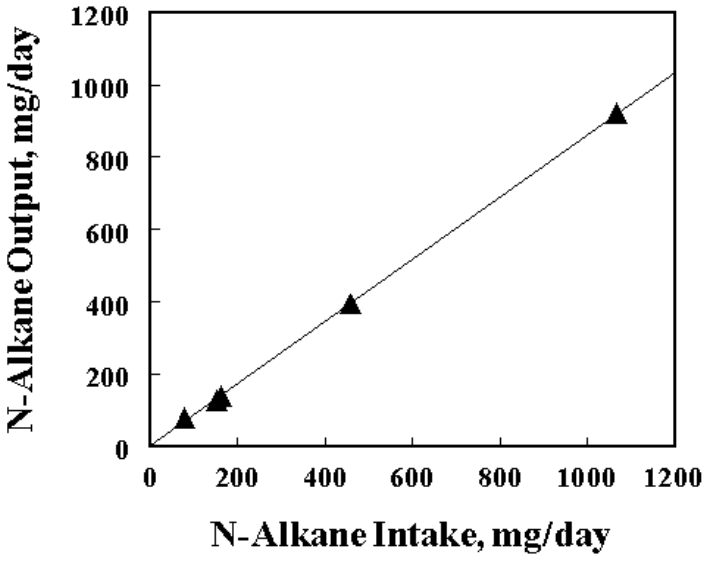

Figure 2. Linear relationship (R_2, %) between mean intake (I_M, mg/d) of all n-alkanes (C25 to C33) and corresponding mean n-alkane fecal output (O_M, mg/d) for eight horses offered tall fescue/alfalfa hay (Diet 1) in Experiment 1

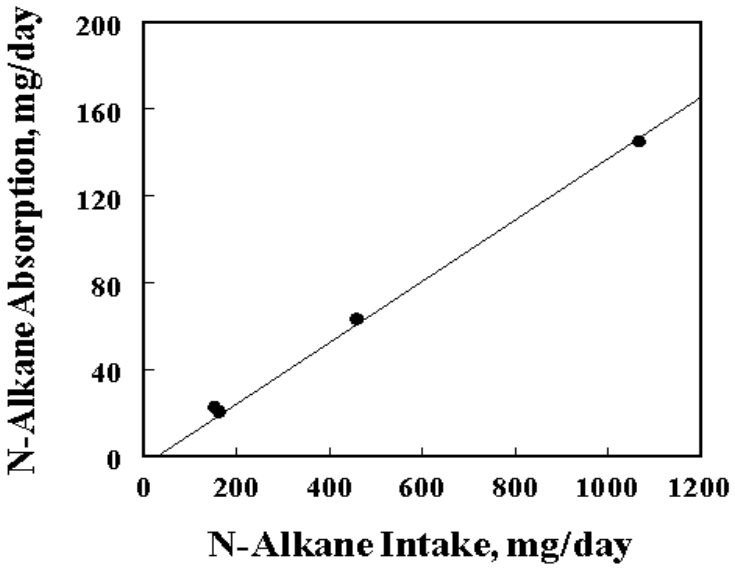

Figure 3. Linear relationship (E_A, %) between mean n-alkane intake (I_M, mg/d) of all n-alkanes (C25 to C33) and corresponding mean n-alkane absorption (A_M, mg/d) for eight horses offered tall fescue/alfalfa hay (Diet 1) in Experiment 1

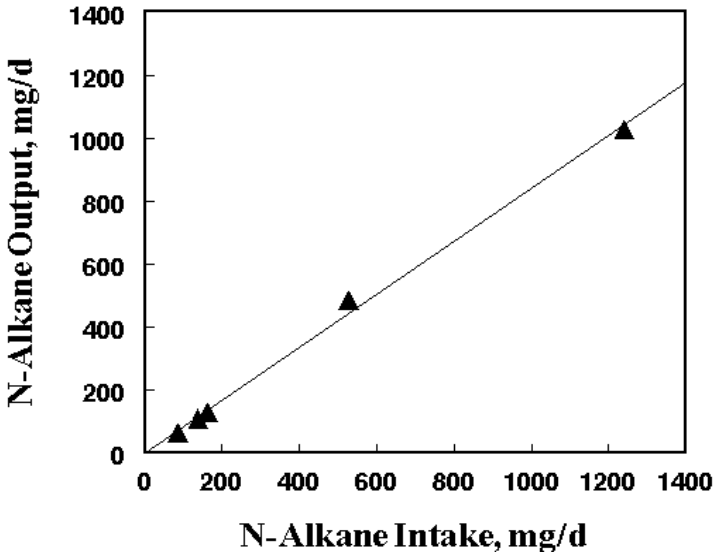

Figure 4. Linear relationship (R_2, %) between mean intake (I_M, mg/d) of all n-alkanes (C25 to C33) and corresponding mean n-alkane fecal output (O_M, mg/d) for eight horses orchardgrass/alfalfa hay (Diet 2) in Experiment 1

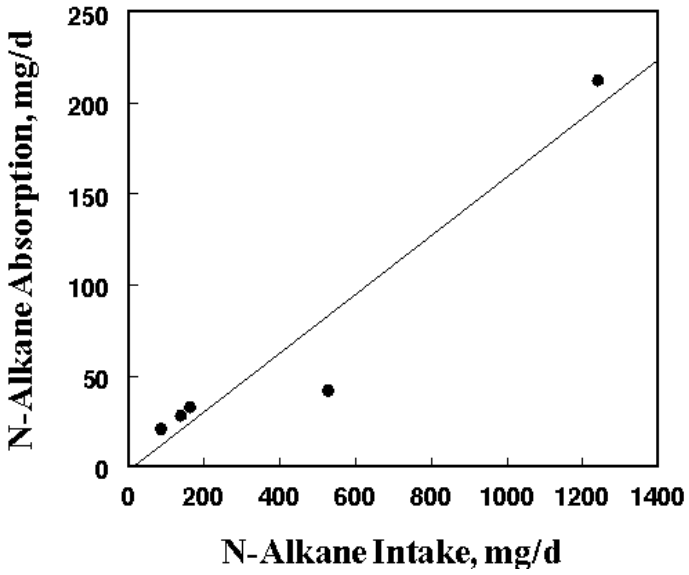

Figure 5. Linear relationship (E_A, %) between mean n-alkane intake (I_M, mg/d) of all n-alkanes (C25 to C33) and corresponding mean n-alkane absorption (A_M, mg/d) for eight horses offered orchardgrass/alfalfa hay (Diet 2) in Experiment 1

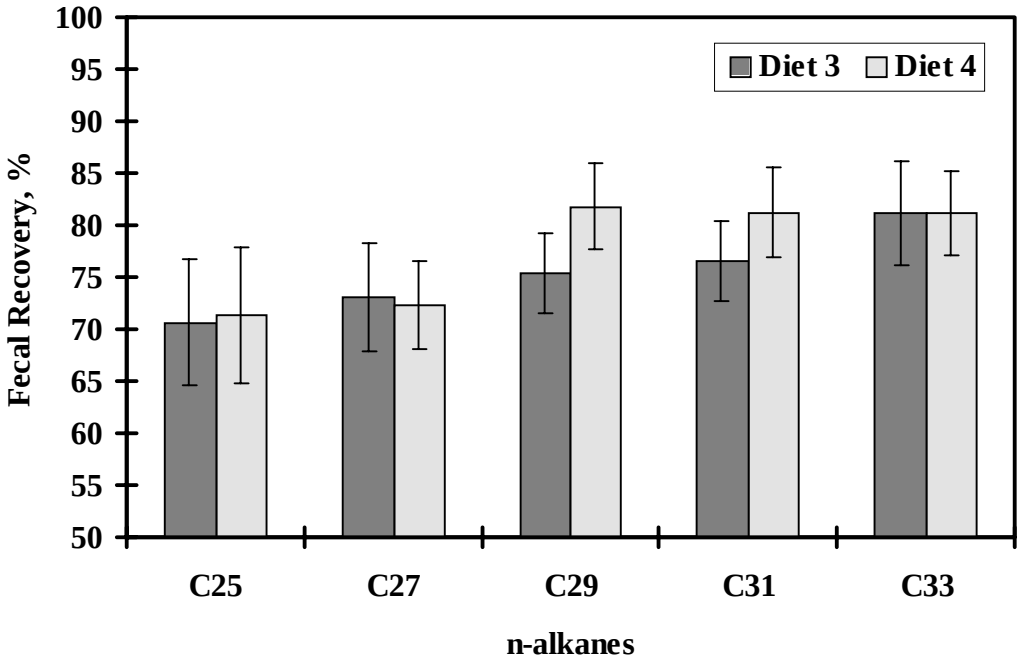

Figure 6. Fecal recovery (mean[a] ± SE[b]) of n-alkanes for horses receiving orchardgrass/alfalfa hay and FF (Diet 3) and orchardgrass/alfalfa hay and SS (Diet 4) in Experiment 2. [a] Mean of Periods 1 and 2; [b] Standard error of the mean (n = 8)

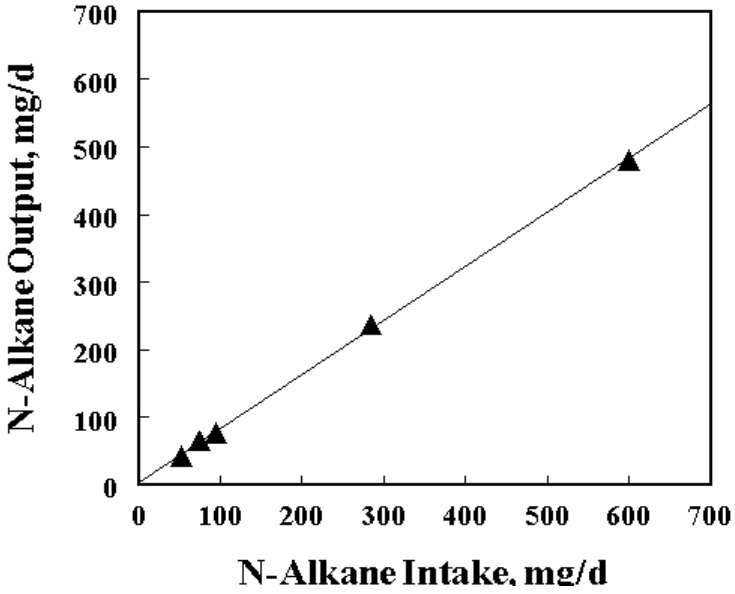

Figure 7. Linear relationship (R_2, %) between mean intake (I_M, mg/d) of all n-alkanes (C25 to C33) and corresponding mean n-alkane fecal output (O_M, mg/d) for eight horses orchardgrass/alfalfa hay and FF (Diet 3) in Experiment 2

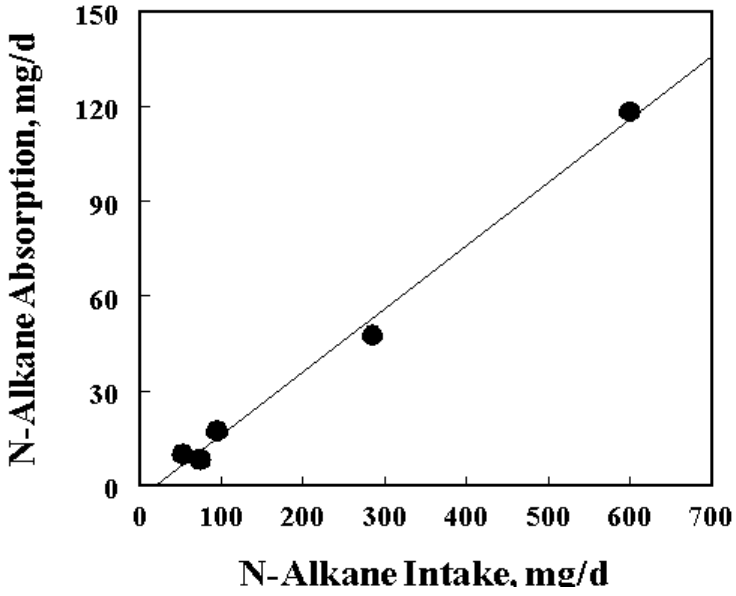

Figure 8. Linear relationship (R_2, %) between mean intake (I_M, mg/d) of all n-alkanes (C25 to C33) and corresponding mean n-alkane fecal output (O_M, mg/d) for eight horses orchardgrass/alfalfa hay and FF (Diet 3) in Experiment 2

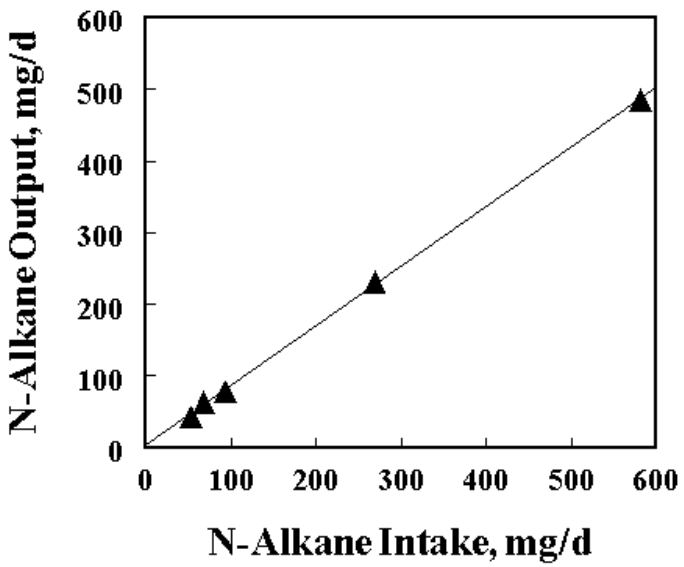

Figure 9. Linear relationship (R_2, %) between mean intake (I_M, mg/d) of all n-alkanes (C25 to C33) and corresponding mean n-alkane fecal output (O_M, mg/d) for eight horses orchardgrass/alfalfa hay and SS (Diet 4) in Experiment 2

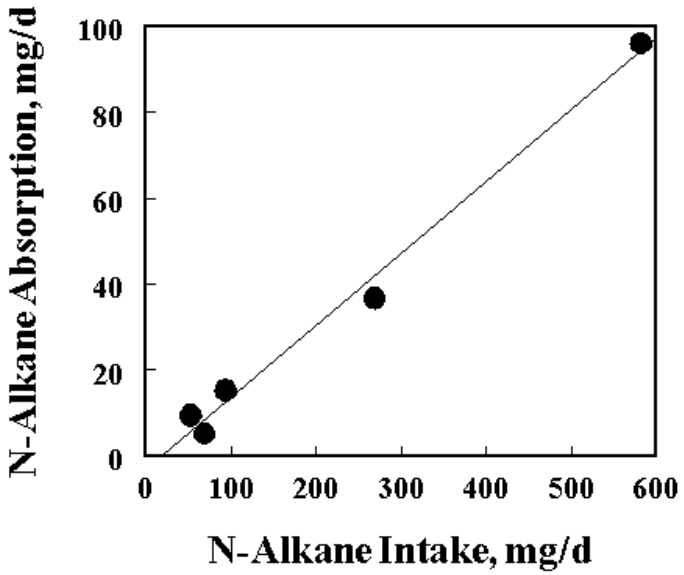

Figure 10. Linear relationship (E_A, %) between mean n-alkane intake (I_M, mg/d) of all n-alkanes (C25 to C33) and corresponding mean n-alkane absorption (A_M, mg/d) for eight horses offered orchardgrass/alfalfa hay and SS (Diet 4) in Experiment 2

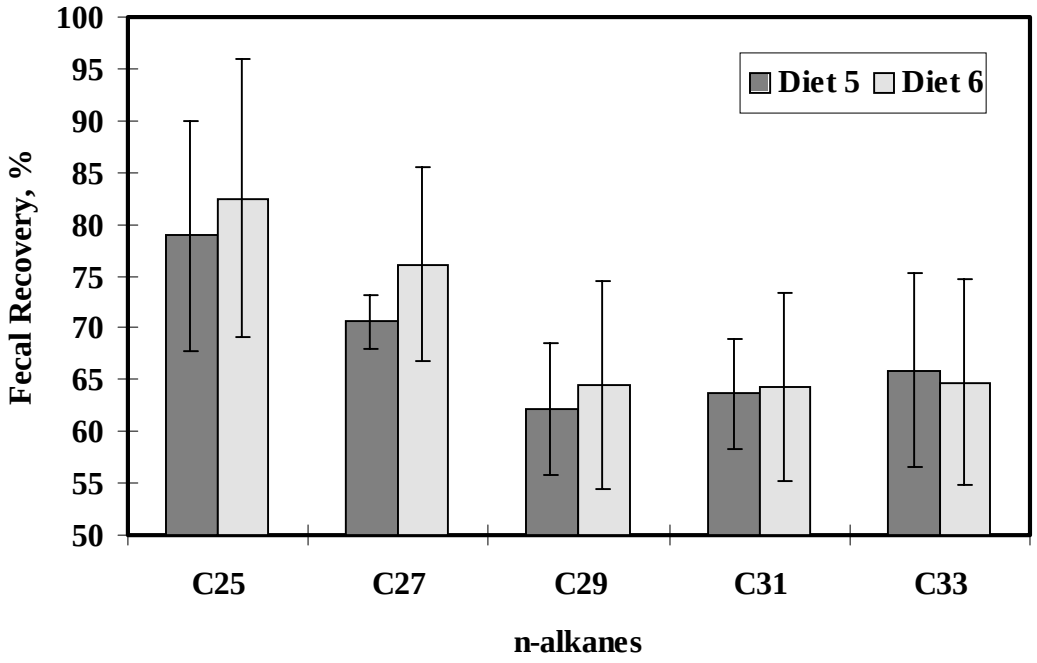

Figure 11. Fecal recovery (mean[a] ± SE[b]) of n-alkanes for stalled horses receiving tall fescue/alfalfa pasture (Diet 5) and bluegrass/white clover pasture (Diet 6) in Experiment 3. [a] Mean of Periods 1 and 2; [b] Standard error of the mean (n = 4)

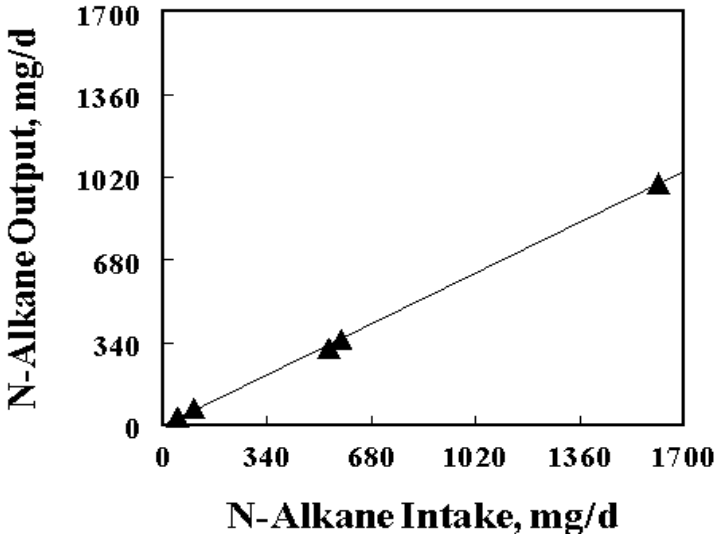

Figure 12. Linear relationship (R_2, %) between mean intake (I_M, mg/d) of all n-alkanes (C25 to C33) and corresponding mean n-alkane fecal output (O_M, mg/d) for four horses tall fescue/alfalfa pasture (Diet 5) in Experiment 3

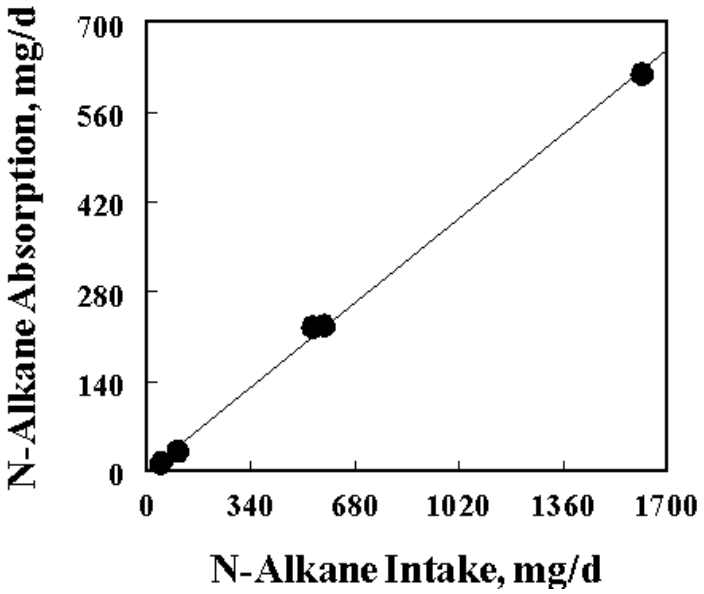

Figure 13. Linear relationship (E_A, %) between mean n-alkane intake (I_M, mg/d) of all n-alkanes (C25 to C33) and corresponding mean n-alkane absorption (A_M, mg/d) for four horses offered tall fescue/alfalfa pasture (Diet 5) in Experiment 3

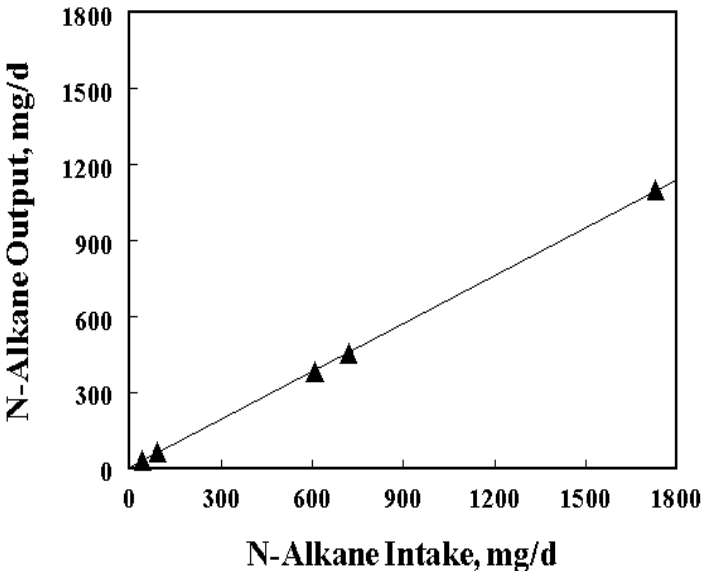

Figure 14. Linear relationship (R_2, %) between mean intake (I_M, mg/d) of all n-alkanes (C25 to C33) and corresponding mean n-alkane fecal output (O_M, mg/d) for four horses bluegrass/white clover pasture (Diet 6) in Experiment 3

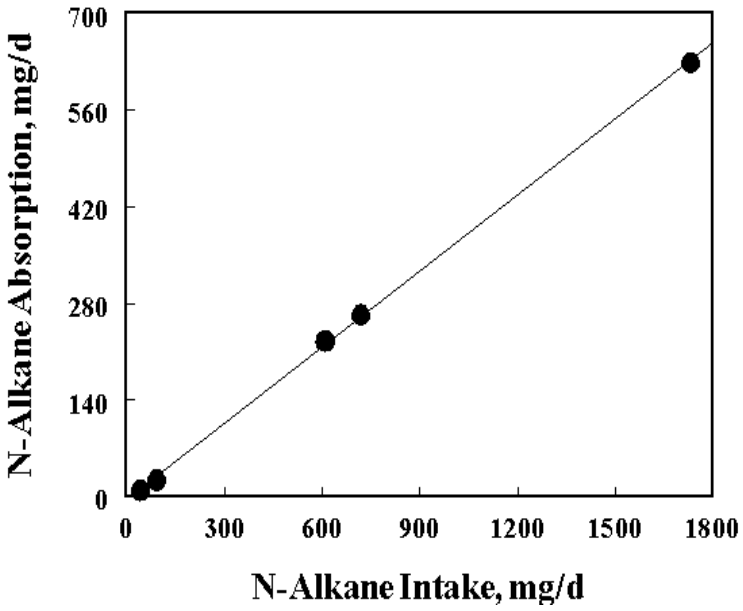

Figure 15. Linear relationship (E_A, %) between mean n-alkane intake (I_M, mg/d) of all n-alkanes (C25 to C33) and corresponding mean n-alkane absorption (A_M, mg/d) for four horses offered bluegrass/white clover pasture (Diet 6) in Experiment 3

Appendix

Appendix table 1. Dietary treatments for horses offered tall fescue/alfalfa hay (Diet 1) or orchardgrass/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 tall fescue/alfalfa (Diet 1) and orchardgrass/alfalfa hay (Diet 2) in Experiment 1[a,b]

	Diet 1		Diet 2	
Item	Mean	SE	Mean	SE
Crude Protein, %	11.8	.54	14.3	.08
Crude Fat, %	1.0	.22	.83	.03
Acid Detergent Fiber, %	48.4	.76	47.5	.85
Neutral Detergent Fiber, %	66.2	1.1	62.6	.58
Non-Structural Carbohydrates, %	13.8	13.8	14.2	.46
Ash, %	7.2	7.2	8.0	.10
TDN, %	53	.41	53	0
DE, Mcal/kg	1.9	.02	2.0	.01
Calcium, %	.61	.02	.72	.02
Phosphorus, %	.25	.003	.30	.01
Magnesium, %	.18	.004	.16	.01
Potassium, %	2.6	.09	3.0	.03
Sodium, %	.012	.002	.01	.002
Iron, mg/kg	645.5	72.30	605.0	114.4
Zinc, mg/kg	36.5	2.60	36.0	3.2
Copper, mg/kg	10.0	.58	10.3	.75
Sulfur, %	.16	.86	.16	.004

[a] Analysis (AOAC, 1990) performed by Dairy One, Ithaca, NY; [b] (n = 4)

Appendix table 3. Dietary treatments for horses offered orchardgrass/alfalfa hay and FF (Diet 1) or orchardgrass/alfalfa hay and SS (Diet 2) 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 (Hay), FF concentrate (Diet 1) and SS concentrate (Diet 2) in Experiment 2[a,b]

	Hay		FF		SS	
Item	Mean	SE	Mean	SE	Mean	SE
Crude Protein, %	13.4	.10	18.4	1.0	14.0	.06
Crude Fat, %	1.9	.15	9.6	1.1	6.9	.15
Acid Detergent Fiber, %	46.5	.94	26.3	1.4	8.7	.16
Neutral Detergent Fiber, %	64.5	1.9	38.1	.50	18.0	.35
Non-Structural Carbohydrates, %	12.5	1.8	25.8	.24	56.0	.27
Ash, %	7.8	.48	8.17	.45	5.1	.04
TDN, %	56	.48	77	1.2	83	.41
DE, Mcal/kg	2.0	.006	2.63	.08	3.6	.009
Calcium, %	.67	.03	1.4	.10	.58	.01
Phosphorus, %	.27	.02	.68	.03	.62	.004
Magnesium, %	.16	.006	.23	.002	.18	.003
Potassium, %	2.5	.24	1.4	.10	.86	.01
Sodium, %	.02	.005	.30	.03	.21	.005
Iron, mg/kg	550.3	139.9	698.8	11.2	605.5	34.4
Zinc, mg/kg	47.5	9.2	164.8	9.7	185.5	3.38
Copper, mg/kg	11.5	1.0	34	2.1	38.0	2.0
Sulfur, %	.15	.002	.20	.004	.19	.006

[a]Analysis (AOAC, 1990) performed by Dairy One, Ithaca, NY; [b] (n = 4)

Appendix table 5. Ingredient composition (%) of the fat and fiber (FF) concentrate 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, 50 mg; Zn, 64 mg; Cu, 20 mg; Mn, 64 mg; Se, .2 mg, I, .1 mg. NaCl used as a carrier (830 g/kg premix).

[b] Courtesy of Hoffman-LaRoche Nutely, NJ

[c] Provided the following amounts per kg of diet: vitamin A, 6,900 IU; β-carotene, 17.6; vitamin D_3, 1,290 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. Nutrient composition of tall fescue/alfalfa pasture (Diet 5) and bluegrass/white clover pasture (Diet 6) in Experiment 3[a]

	Diet 5		Diet 6	
Item	Mean	SE	Mean	SE
Crude Protein, %	12.2	.89	12.6	.44
Crude Fat, %	3.1	.38	3.1	.19
Acid Detergent Fiber, %	42.1	.93	40.5	.96
Neutral Detergent Fiber, %	63.8	.49	66.9	1.5
Non-Structural Carbohydrates, %	11.5	.64	10.3	1.1
Ash, %	9.39	.04	7.0	.18
TDN, %	57.8	.48	59.3	.25
DE, Mcal/kg	1.96	.04	2.01	.04
Calcium, %	.91	.07	.67	.05
Phosphorus, %	.31	.004	.30	.005
Magnesium, %	.31	.02	.18	.003
Potassium, %	2.1	.22	1.8	.25
Sodium, %	.01	.003	.004	.001
Iron, mg/kg	316.3	45.6	481.3	20.6
Zinc, mg/kg	35.3	1.0	39.3	2.5
Copper, mg/kg	5	.41	5.3	.63
Sulfur, %	.24	.004	.18	.01

[a]Analysis (AOAC, 1990) performed by Dairy One, Ithaca, NY; [b] (n = 4)

Appendix table 7. Dietary and condition treatments for horses offered tall fescue/alfalfa pasture (Diet 5) or bluegrass/white clover pasture (Diet 6) in Experiment 3

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

[a] June 1997
[b] August 1997

Appendix table 8. Dry matter intake (DMI, kg/day), total fecal output (FO, kg/day), and total collection DMD (D_{TC}, %) for horses offered tall fescue/alfalfa hay (Diet 1) and orchardgrass/alfalfa hay (Diet 2) in Experiment 1

	Diet 1			Diet 2		
Horse	DMI	FO	D_{TC}	DMI	FO	D_{TC}
1	9.34	4.73	49.36	9.60	3.87	59.69
2	11.01	4.68	57.49	10.09	3.40	66.30
3	6.77	3.15	53.47	8.10	3.11	61.60
4	9.83	4.38	55.44	11.59	4.75	59.02
5	11.10	5.46	50.81	11.30	5.03	55.49
6	8.18	3.98	51.34	11.45	4.11	64.10
7	7.53	3.21	57.37	7.90	2.85	63.92
8	11.22	4.81	57.13	11.38	4.90	56.94
Mean ± SE[a]	9.38 ± .61 [b]	4.30 ± .29 [c]	54.05 ± 1.15 [d]	10.18 ± .54[b]	4.00 ± .30 [c]	60.88 ± 1.33 [d]

[a] Standard error of the mean (n = 8)
[b] (P = .956); [c] (P = .966); [d] (P = .015)

Appendix table 9. N-alkane concentrations (mg/kg DM) in tall fescue/alfalfa (Diet 1) and orchardgrass/alfalfa hay (Diet 2) offered in Experiment 1[a]

	n-alkanes				
Diet	C25	C27	C29	C31	C33
Diet 1					
Mean ± SE[b]	9.66 ± 1.41[c]	15.95 ± 1.85[d]	48.54 ± 2.62[e]	114.12 ± 1.87[f]	16.98 ± 1.56[g]
Diet 2					
Mean ± SE	9.00 ± .48[c]	15.82 ± .58[d]	51.85 ± .51[e]	121.89 ± .66[f]	13.49 ± .77[g]

[a] Periods 1 and 2
[b] Standard error of the mean (n = 2)
[c] (P = .703); [d] (P = .949); [e] (P = .341); [f] (P = .059); [g] (P = .183)

Appendix table 10. Fecal concentrations (mg/kg DM) of n-alkanes for horses offered tall fescue/alfalfa (Diet 1) and orchardgrass/alfalfa hay (Diet 2) in Experiment 1

	Diet 1				
Horse	C25	C27	C29	C31	C33
1	22.35	31.85	93.67	223.92	33.91
2	18.38	30.73	94.48	211.87	32.63
3	19.63	32.44	107.53	256.54	34.75
4	17.39	29.15	88.45	200.86	33.97
5	15.73	26.92	83.43	186.89	29.84
6	18.68	30.81	91.71	233.19	32.12
7	22.53	35.38	106.97	241.00	34.85
8	16.29	26.22	80.49	190.13	31.40
Mean ± SE[a]	18.87 ± .90[b]	30.44 ± 1.06[c]	93.34 ± 3.48[d]	218.05 ± 8.82[e]	33.06 ± .68[f]

	Diet 2				
Horse	C25	C27	C29	C31	C33
1	15.21	31.84	127.43	264.77	28.82
2	18.80	35.24	119.30	256.39	29.84
3	14.98	29.81	118.69	250.86	26.39
4	16.33	30.84	114.16	237.90	26.72
5	16.56	33.77	123.91	261.69	26.22
6	14.10	29.85	124.11	266.73	26.96
7	16.81	34.70	128.37	260.00	28.19
8	16.07	31.80	116.92	257.42	27.02
Mean ± SE	16.11 ± .50[b]	32.23 ± .75[c]	121.61 ± 1.81[d]	256.97 ± 3.24[e]	27.52 ± .46[f]

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

[b] (P = .039); [c] (P = .186); [d] (P = .0001); [e] (P = .003); [f] (P = .0001)

Appendix table 11. Dry matter intake (DMI, kg/day), total fecal output (FO, kg/day), 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 Concentrate	FO	D_{TC}	DMI Hay	DMI Concentrate	FO	D_{TC}
1	5.79	2.15	3.54	55.42	5.81	1.95	3.51	54.77
2	5.90	2.02	3.08	61.11	6.28	2.15	3.34	60.38
3	5.46	1.89	3.29	55.24	5.53	1.95	2.99	60.03
4	4.95	1.64	2.61	60.39	4.96	1.79	3.05	54.81
5	5.44	2.02	3.54	52.55	5.46	1.79	3.43	52.69
6	5.46	1.64	2.71	61.83	4.70	1.83	2.32	64.47
7	4.63	1.64	2.77	55.82	4.70	1.83	2.35	64.01
8	4.95	1.64	2.71	58.88	4.96	1.79	3.42	49.33
Mean ± SE[a]	5.32 ± .16[b]	1.83 ± .08[c]	3.03 ± .14[d]	57.66 ± 1.18[e]	5.30 ± .20[b]	1.88 ± .04[c]	3.05 ± .17[d]	57.56 ± 1.94[e]

[a] Standard error of the mean (n = 8)
[b] (*P* = .849); [c] (*P* = .395); [d] (*P* = .894); [e] (*P* = .387)

Appendix table 12. N-alkane concentration (mg/kg DM) in orchardgrass/alfalfa hay, Fat and Fiber (FF), and Sugar and Starch (SS) concentrates offered in Experiment 2[a]

	n-alkanes				
Diet Component	C25	C27	C29	C31	C33
Orchardgrass/alfalfa hay					
Mean ± SE[b]	9.35 ± .76	16.61 ± .74	49.56 ± 1.13	108.56 ± .59	12.87 ± .29
FF					
Mean ± SE	7.25 ± 1.7	9.80 ± 1.67	26.68 ± .69	26.51 ± 2.29	6.77 ± 1.01
SS					
Mean ± SE	8.24 ± .17	10.55 ± .04	10.26 ± .02	9.91 ± .21	4.83 ± .26

[a] Each observation represents mean of Periods 1 and 2
[b] Standard error of the mean (n = 2)

Appendix table 13. N-alkane concentration (mg/kg DM) in orchardgrass/alfalfa hay and FF (Diet 3) and orchardgrass/alfalfa hay and SS (Diet 4) offered in Experiment 2[a]

	Diet 3					Diet 4				
	n-alkanes					n-alkanes				
Horse	C25	C27	C29	C31	C33	C25	C27	C29	C31	C33
1	8.78	14.77	43.37	86.36	11.22	9.07	15.09	39.69	83.80	10.85
2	8.81	14.88	43.73	87.65	11.31	9.06	15.07	39.54	83.42	10.82
3	8.81	14.86	43.66	87.42	11.30	9.06	15.03	39.31	82.84	10.77
4	8.82	14.92	43.86	88.15	11.35	9.05	15.00	39.13	82.39	10.74
5	8.78	14.77	43.37	86.38	11.22	9.07	15.11	39.84	84.18	10.88
6	8.86	15.04	44.26	89.58	11.46	9.04	14.92	38.56	80.95	10.62
7	8.80	14.83	43.57	87.08	11.27	9.04	14.92	38.56	80.95	10.62
8	8.82	14.92	43.86	88.15	11.35	9.05	15.00	39.13	82.39	10.74
Mean ± SE[b]	8.81 ± .01	14.87 ± .03	43.71 ± .10	87.60 ± .37	11.31 ± .03	9.05 ± .01	15.02 ± .03	39.22 ± .17	82.61 ± .43	10.76 ± .03

[a] Concentrations weighted for hay (74 %) and concentrate (26 %) intake
[b] Standard error of the mean (n = 2)

Appendix table 14. Fecal n-alkane concentration (mg/kg DM) for horses offered orchardgrass/alfalfa hay and FF concentrate (Diet 3) and orchardgrass/alfalfa hay and SS (Diet 4) in Experiment 2

	Diet 3					Diet 4				
	n-alkanes					n-alkanes				
Horse	C25	C27	C29	C31	C33	C25	C27	C29	C31	C33
1	14.39	27.23	91.10	182.14	24.07	14.63	28.31	90.37	196.71	23.48
2	15.20	27.29	81.16	172.19	22.27	12.87	23.98	75.94	152.90	18.73
3	14.92	26.80	80.38	154.14	21.93	15.46	28.60	86.38	187.32	23.18
4	12.54	22.24	63.47	132.92	18.20	13.98	24.49	67.47	137.80	20.33
5	13.21	23.93	74.65	155.25	20.41	14.73	25.50	72.82	146.17	18.83
6	11.62	22.52	76.89	161.50	21.30	13.22	24.81	75.36	169.40	21.59
7	15.66	27.48	81.73	157.64	22.70	13.33	24.18	74.81	158.22	19.47
8	14.14	24.53	71.27	146.61	20.85	13.75	24.08	64.08	127.13	19.39
Mean ± SE[a]	13.96 ± .49[b]	25.25 ± .78[c]	77.58 ± 2.89[d]	157.80 ± 5.31[e]	21.46 ± .62[f]	14.00 ± .31[b]	25.49 ± .67[c]	75.90 ± 3.11[d]	159.45 ± 8.45[e]	20.62 ± .67[f]

[a] Standard error of the mean (n = 8)
[b] (*P* = .951); [c] (*P* = .780); [d] (*P* = .357); [e] (*P* = .799); [f] (*P* = .278)

Appendix table 15. Dry matter intakes (DMI, kg/day), total fecal outputs (FO, kg/day), and total collection digestibility (D_{TC}, %) for stalled horses offered tall fescue/alfalfa pasture (Diet 5) and bluegrass/white clover pasture (Diet 6) in Experiment 3

	Diet 5			Diet 6		
Horse	DMI	FO	D_{TC}	DMI	FO	D_{TC}
1	9.26	3.33	64.04	9.15	2.67	70.82
2	11.63	4.00	65.61	9.79	3.80	61.18
3	9.46	3.19	66.28	6.79	2.62	61.41
4	12.86	4.07	68.35	11.05	4.18	62.18
Mean ± SE[a]	10.80 ± .87[b]	3.65 ± .23[c]	66.07 ± .89[d]	9.19 ± .89[b]	3.23 ± .34[c]	63.47 ± 1.85[d]

[a] Standard error of the mean (n = 4)
[b] (*P* = .418); [c] (*P*=.450); [d] (*P* = .854)

Appendix table 16. N-alkane concentration (mg/kg DM) in tall fescue/alfalfa pasture (Diet 5) and bluegrass/white clover pasture (Diet 6) offered in Experiment 3[a]

	n-alkanes				
Diet	C25	C27	C29	C31	C33
Diet 5					
Mean ± SE[b]	4.30 ± 1.76[c]	9.54 ± 3.71[d]	48.61 ± 11.23[e]	139.69[f] ± 4.81[e]	40.49 ± 3.23[g]
Diet 6					
Mean ± SE	4.44 ± 1.33[c]	9.63 ± 4.02[d]	76.18 ± 17.64[e]	186.52 ± 17.64[f]	67.19 ± 12.31[g]

[a] Each observation represents mean of Periods 1 and 2
[b] Standard error of the mean (n = 2)
[c] (*P* = .951); [d] (*P* = .987); [e] (*P* = .362); [f] (*P* = 124); [g] (*P* = .102)

Appendix table 17. N-alkane concentration (mg/kg DM) in individual components of tall fescue/alfalfa pasture (Diet 5) and bluegrass/white clover pasture (Diet 6) offered in Experiment 3[a]

	n-alkanes				
Component	C25	C27	C29	C31	C33
Diet 5					
Tall Fescue	2.87 ± .26	7.30 ± .56	47.91 ± 2.43	151.05 ± 7.24	43.33 ± 4.66
Alfalfa	2.14 ± .90	12.30 ± 1.15	63.70 ± 5.73	209.38 ± 36.62	18.25 ± 4.45
Diet 6					
Bluegrass	6.37 ± 1.02	13.56 ± 2.26	108.08 ± 8.40	285.98 ± 5.32	108.11 ± 6.27
White Clover	4.63 ± .94	13.38 ± 2.51	71.30 ± 9.68	117.0 ± 13.65	18.14 ± .92

[a] Each observation represents mean ± SE of Periods 1 and 2 (n = 2)

Appendix table 18. Fecal concentration (mg/kg DM) of n-alkanes for stalled and pastured horses offered tall fescue/alfalfa pasture (Diet 5) in Experiment 3

	Diet 5				
Horse	n-alkanes				
Stalled	C25	C27	C29	C31	C33
1	6.31	11.89	68.11	263.26	87.10
2	10.16	24.23	111.90	264.17	77.52
3	11.38	28.24	132.16	310.41	90.16
4	8.46	13.18	78.09	262.28	93.33
Mean ± SE[a]	9.08 ± 1.10[b]	19.39 ± 4.05[c]	97.56 ± 14.86[d]	275.03 ± 11.80[e]	87.03 ± 3.42[f]
Pasture					
5	42.05	47.54	126.05	374.86	108.01
6	73.57	142.07	142.91	418.14	105.99
7	105.50	92.33	208.32	374.90	69.14
8	115.59	177.20	216.40	395.87	70.06
Mean ± SE	84.18 ± 16.65[b]	114.78 ± 28.38[c]	173.42 ± 22.80[d]	390.94 ± 10.33[e]	88.30 ± 10.81[f]

[a] Standard error of the mean (n = 4)
[b] (P = .018); [c] (P = .044); [d] (P = .044); [e] (P = .009); [f] (P = .931)

Appendix table 19. Fecal concentration (mg/kg DM) of n-alkanes for stalled and pastured offered bluegrass/white clover pasture (Diet 6) in Experiment 3

	Diet 6				
Horse	n-alkanes				
Stalled	C25	C27	C29	C31	C33
1	14.23	32.64	193.41	417.81	121.30
2	6.84	11.83	84.49	258.80	109.01
3	7.52	11.33	98.17	277.56	121.62
4	11.96	27.77	179.52	399.85	123.09
Mean ± SE[a]	10.13 ± 1.78[b]	20.90 ± 5.47[c]	138.89 ± 27.75[d]	338.50 ± 40.95[e]	118.75 ± 3.27[f]
Pasture					
5	87.72	72.79	261.87	437.70	104.99
6	48.60	59.57	228.93	427.00	120.75
7	64.36	69.83	113.96	302.65	127.57
8	74.90	88.16	119.93	295.26	116.35
Mean ± SE	68.89 ± 8.28[b]	72.59 ± 5.91[c]	181.17 ± 37.71[d]	365.65 ± 38.60[e]	117.41 ± 4.74[f]

[a] Standard error of the mean (n = 4)
[b] (P = .003); [c] (P = .001); [d] (P = .398); [e] (P = .659); [f] (P = .845)

Vita

Amy L. Ordakowski

Amy Lynn Ordakowski, daughter of Mrs. Carol Slicher and Mr. Paul Ordakowski, was born on March 6, 1973 in Baltimore, Maryland. She graduated from Chesapeake High School in Pasadena, Maryland in 1991, then attended James Madison University majoring in Biology. The author was awarded her Bachelor of Science degree in May, 1995. Following her undergraduate degree, she pursued graduate studies in the Department Animal and Poultry Sciences at Virginia Polytechnic Institute and State University in Equine Nutrition where she was a member of Phi Sigma National Biological Honor Society. She was awarded a John L. Pratt Fellowship to pursue a doctoral degree in Equine Nutrition from Virginia Polytechnic Institute and State University.