Fat digestibility in *Equus caballus* follows increasing first-order kinetics


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**ABSTRACT:** The digestibility of ether extract varies greatly from forages to grains and further to added fats consisting mainly of triglycerides. This variation has been attributed to two main factors, the presence of nonhydrolyzable substances in the ether extract, especially in leafy foods, and the dilution of endogenous fecal fat. A compilation of results from 188 equine digestion balance observations on five basal feeds and 18 test feeds with added fats demonstrated a true digestibility of fat approaching 100% and an endogenous fecal fat of 0.22 g H\(^{-1}\) kg BW\(^{-1}\). The results revealed that nonhydrolyzable ether extract and endogenous fecal fat were insufficient to account for the difference between true digestibility and apparent digestibilities of ether extract in basal feeds and partial digestibilities of added fats in test feeds. A third possible contributing factor was demonstrated: an increasing first-order relationship between observed digestibilities (D, %) and the fat content of the feed (F, g/kg): D = 92.0 - 92.0e\(^{-F/342}\), \(r^2 = 0.81, P < 0.001\). This equation indicates that 46% digestibility (half maximum) occurs at an ether extract or fat content of 24 g/kg, which is common in forages. It is consistent with fat digestibility or efficiency of absorption being a function of the rate of lipolysis, especially when residence time in the small intestine is limited. Consequently, we suggest that the kinetics of lipases, which are difficult to measure, may contribute to low digestibility when substrate concentration in the small intestine is low due to a low fat content in food. The status of vitamins A and E might be affected by low dietary fat contents and might be improved by fat supplementation.

Key Words: Digestibility, Enzyme Kinetics, Fats, Horses, Lipase


**Introduction**

Mean estimates of apparent digestibilities (\(D_a\)) of ether extract (EE) in horses and ponies are 42 to 49% for forages (Fonnesbeck et al., 1967; Bowman et al., 1979; Sturgeon et al., 2000), 55 to 76% for grains (Hintz and Schryver, 1989), and 88 to 94% for added fats and oils consisting almost entirely of triglycerides (Kane et al., 1979; McCann et al., 1987). The low \(D_a\) of EE in forages is usually attributed to the presence of waxes, sterols, pigments, and other indigestible lipids (Van Soest, 1994).

In this article, we have compiled the results of digestibility balance experiments on 23 feeds that were included in three theses (Bowman, 1977; Custalow, 1992; Holland, 1994) and one dissertation (Rich, 1980). The number of feeds enabled the testing of the hypotheses that true digestibility (\(D_t\)) of fat approaches 100%, and that endogenous fecal fat (\(F_{ef}\)) can account for the difference between \(D_t\) and \(D_a\) of added triglycerides and part of the difference for forages.

**Materials and Methods**

Digestibility balance experiments were conducted on 25 ponies and 16 light horses at the Smithfield Horse Center. The protocol was approved by the institutional animal care and use committee. Experiments on 23 feeds were grouped into five trials, each with a basal feed plus at least one feed with added corn oil and up to seven other feeds with added fats. The present analysis was based on the mean apparent digestibilities of DM and nutrients in the 23 feeds.

**Animals**

Trials 1 to 3 used eight, eight, and nine mature pony geldings, respectively (Bowman, 1977; Rich, 1980).
Table 1. Formulas of basal diets, % as-fed basis

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
<th>Trial 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa hay</td>
<td>54.7</td>
<td>23.3</td>
<td>29.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Orchardgrass hay</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>48.0</td>
<td>—</td>
</tr>
<tr>
<td>Mixed grass hay</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>58.4</td>
</tr>
<tr>
<td>Corn, grain</td>
<td>26.4</td>
<td>23.3</td>
<td>26.5</td>
<td>43.0</td>
<td>13.5</td>
</tr>
<tr>
<td>Oats, grain</td>
<td>19.0</td>
<td>23.3</td>
<td>26.5</td>
<td>—</td>
<td>13.4</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>—</td>
<td>11.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Corn cobs, ground</td>
<td>—</td>
<td>8.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sugar cane molasses</td>
<td>—</td>
<td>8.8</td>
<td>—</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Cane molasses, dehydrated</td>
<td>—</td>
<td>—</td>
<td>4.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Beet pulp, sugar, dehydrated</td>
<td>—</td>
<td>—</td>
<td>11.8</td>
<td>—</td>
<td>6.2</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.01</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Limestone</td>
<td>—</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Vitamin-mineral premixa</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Chromic sesquioxide</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Equi-Choice vitamin mineral supplement (Wilson Enterprises, Inc., Disputanta, VA). Guaranteed analysis: Ca (maximum), 9.0%; Ca (minimum), 7.0%; P, 8.0%; Mg, 1.0%; S, 0.5%; Fe, 0.3%; Cu, 0.12%; Mn, 0.1%; Co, 0.002%; NaCl (maximum), 36.0%; NaCl (minimum), 34.0%; Na, 13.65%; Cl, 21.35%; Se, 0.0011%; vitamin A, 330,000 IU/kg; vitamin D, 55,000 IU/kg.

Trial 4 used eight mature Arabians, five fillies, and three geldings (Custalow, 1992). Trial 5 used four geldings and four fillies of mixed light breeds between 2 and 7 yr old (Holland, 1994). In each trial, all animals were rotated randomly through all feeds in successive periods so that each horse received each diet for one period, giving a total of 188 balance observations. The ponies weighed 235 ± 3 kg, horses, 374 ± 22 kg, and the average for both was 289 ± 16 kg.

Animals were kept in approximately 3 × 3 m stalls. They were allowed access to a dirt paddock for free exercise for part of the day. In Trial 4, horses were walked 4 d/wk on a treadmill (Mustang 2200; Kagra AG, 5615 Fahrwangen, Switzerland).

Animals were individually fed their daily ration divided into two equal meals. Daily intakes (DM basis) were 3.91 ± 0.16 kg/d for ponies and 5.99 ± 0.61 kg/d for horses. Feeds were introduced gradually over 7 d to avoid shiny, loose, or greasy stools, and further accommodation was allowed for 14 d.

**Feeds**

The five basal feeds were formulated to meet or exceed recommended nutrient requirements (NRC, 1973, 1989). These complete feeds contained 42.8 ± 6.9% hays and 43.0 ± 4.3% grains (as-fed basis; Table 1). Fats or oils were included in 18 test feeds. Trial 1 compared basal feed with three feeds containing 5, 10, or 20% added corn oil by weight as fed. Trial 2 compared basal feed with eight feeds containing 7.5 or 15% added corn oil, peanut oil, animal-vegetable blend, or inedible tallow. Trial 3 compared basal feed with feeds containing 10% added corn oil, tallow, or blend. Trial 4 compared basal feed with feeds containing 10% corn oil. Trial 5 compared basal feed with feeds containing 10% corn oil, 1:1 mixtures of soy lecithin and corn oil, or soy lecithin and soybean oil. Chemical analysis of feeds was performed in the Virginia Tech Dairy Herd Improvement Association Laboratory (Table 2).

**Balance Experiments**

In balance trials, each meal was weighed, and orts were collected just before the next meal and weighed. In Trials 1 to 3, total feces were collected in fecal collection bags, weighed, and samples were taken. In Trials 4 and 5, chromic sesquioxide was given as a marker, and fecal grab samples were taken every 12 h during the collection period (Holland et al., 1998). After 21 d of introduction and accommodation, feed and fecal samples were collected for 7 d, combined, and mixed thoroughly. Combined samples were taken in duplicate and frozen for subsequent analysis. Feeds and feces were analyzed for DM, CP, EE (AOAC, 1990), and ADF (Goering and Van Soest, 1970).

**Calculations**

Digestibilities were calculated according to Kleiber (1961). For total collection experiments, $D_a$ is a function of intake ($I$) and fecal output ($O$):

$$D_a = 1 - (O/I)$$

For marker experiments, $D_a$ is a function of marker concentrations in intake ($M_i$) and feces ($M_o$):

$$D_a = 1 - (M_i/M_o)$$

True digestibility of fat was calculated as the slope and $F_{ef}$ as the intercept when absorbed fat ($F_a = F_i - F_o$, g/d) was plotted against fat intake ($F_i$, g/d).

Partial digestibilities ($D_p$) of added fats were calculated from increments (i.e., the increase in $D_a$ attrib-
Fat absorption kinetics

Table 2. Chemical analysis of feeds

<table>
<thead>
<tr>
<th>Item</th>
<th>DM, g/kg</th>
<th>EE a</th>
<th>CP</th>
<th>ADF b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>903</td>
<td>37</td>
<td>141</td>
<td>233</td>
</tr>
<tr>
<td>Corn oil, 5%</td>
<td>915</td>
<td>76</td>
<td>130</td>
<td>219</td>
</tr>
<tr>
<td>Corn oil, 10%</td>
<td>925</td>
<td>128</td>
<td>109</td>
<td>207</td>
</tr>
<tr>
<td>Corn oil, 20%</td>
<td>935</td>
<td>233</td>
<td>97</td>
<td>232</td>
</tr>
<tr>
<td><strong>Trial 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>884</td>
<td>35</td>
<td>133</td>
<td>166</td>
</tr>
<tr>
<td>Corn oil, 7.5%</td>
<td>890</td>
<td>102</td>
<td>126</td>
<td>168</td>
</tr>
<tr>
<td>Corn oil, 15%</td>
<td>890</td>
<td>184</td>
<td>115</td>
<td>167</td>
</tr>
<tr>
<td>Peanut oil, 7.5%</td>
<td>900</td>
<td>113</td>
<td>123</td>
<td>199</td>
</tr>
<tr>
<td>Peanut oil, 15%</td>
<td>905</td>
<td>190</td>
<td>114</td>
<td>195</td>
</tr>
<tr>
<td>Tallow, 7.5%</td>
<td>886</td>
<td>112</td>
<td>125</td>
<td>186</td>
</tr>
<tr>
<td>Tallow, 15%</td>
<td>907</td>
<td>191</td>
<td>115</td>
<td>167</td>
</tr>
<tr>
<td>Blend, 7.5%</td>
<td>880</td>
<td>105</td>
<td>129</td>
<td>161</td>
</tr>
<tr>
<td>Blend, 15%</td>
<td>903</td>
<td>188</td>
<td>116</td>
<td>171</td>
</tr>
<tr>
<td><strong>Trial 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>890</td>
<td>33</td>
<td>134</td>
<td>184</td>
</tr>
<tr>
<td>Corn oil, 10%</td>
<td>903</td>
<td>134</td>
<td>122</td>
<td>165</td>
</tr>
<tr>
<td>Blend, 10%</td>
<td>901</td>
<td>138</td>
<td>122</td>
<td>160</td>
</tr>
<tr>
<td>Tallow, 10%</td>
<td>900</td>
<td>139</td>
<td>122</td>
<td>163</td>
</tr>
<tr>
<td><strong>Trial 4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>893</td>
<td>23</td>
<td>100</td>
<td>318</td>
</tr>
<tr>
<td>Corn oil, 10%</td>
<td>901</td>
<td>157</td>
<td>93</td>
<td>340</td>
</tr>
<tr>
<td><strong>Trial 5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>891</td>
<td>26</td>
<td>113</td>
<td>276</td>
</tr>
<tr>
<td>Corn oil, 10%</td>
<td>913</td>
<td>100</td>
<td>105</td>
<td>267</td>
</tr>
<tr>
<td>Soy lecithin, 5% + corn oil, 5%</td>
<td>915</td>
<td>102</td>
<td>105</td>
<td>274</td>
</tr>
<tr>
<td>Soy lecithin, 5% + soybean oil, 5%</td>
<td>915</td>
<td>101</td>
<td>105</td>
<td>278</td>
</tr>
</tbody>
</table>


uted to the added fat), as demonstrated by Kleiber (1961), who differentiated between apparent and partial digestibilities in regard to added materials, such as fat. Using data from Trial 4, we can provide a numerical example. Given that the basal feed has 23 g/kg of fat and its Da is 51%, and the basal+corn oil feed has 157 g/kg of fat and its DaB+C is 86%, then Dp can be solved as follows:

\[
D_{aB+C} = \frac{D_a}{23 / 157} + D_p \frac{(157 - 23) / 157};
\]

thus Dp = 92%.

Statistical Analysis

Data from individual animals were summarized as means and standard errors of 23 feeds in the original five trials or sets of experiments (Bowman, 1977; Rich, 1980; Custalow, 1992; Holland, 1994), and these 23 means of feeds were used in the present analysis. Apparent digestibilities for five basal and 18 test feeds were compared with the t-test; an alpha level of \( P < 0.05 \) was used for determination of significance, with an alpha level of \( P < 0.10 \) used for the determination of statistical trend. Estimates of D_t and F_ef were obtained by a regression of F_a on F_I, with the slope represented by D_t and the negative intercept representing F_ef (Palmquist, 1991). Evaluation of first-order kinetics, estimates of asymptotic or maximal values for D_a and D_p, and the dietary fat concentration (F_c, g/kg of DM of diet) at half maximal digestibility were obtained by analogy with enzyme kinetics (Smith et al., 1983).

Graphical analysis assessed goodness of fit as \( r^2 \) and adjusted \( r^2 \) with a significant \( P \)-value and no systematic deviations (SlideWritePlus, Advanced Graphics Software, Encinitas, CA).

Results

All animals remained healthy throughout these experiments. Daily DMI were 5.09 ± 0.83 kg for five basal feeds and 4.28 ± 0.22 kg for 18 test feeds. Apparent digestibilities of EE were lower (\( P < 0.001 \)) for the basal feeds than for the test feeds (55 ± 5 and 81 ± 2%, respectively), and corresponding EE contents were 31 ± 3 and 138 ± 19 g/kg. No trend for an associative effect was found overall; hence, \( D_a \) estimates are summarized for basal feeds and test feeds together: DM, 68 ± 2%; CP, 72 ± 2%; ADF, 55 ± 3%. The \( D_a \) of ADF was 56.1 ± 8.4% in basal feeds and 54.8 ± 3.6% in test feeds (\( P = 0.89 \)).

Partial digestibility was 87 ± 2% for corn oil and 90 ± 2% for all other fats and oils. Partial digestibility
Figure 1. Relationship between dietary fat content and digestibility. Digestibility of fats increased exponentially to an asymptotic value of 95% for added fats in 18 test feeds (dashed line; \( r^2 = 0.50, P < 0.001 \)) or 92% for all 23 feeds (solid line; \( r^2 = 0.81, P < 0.001 \)), including five basal feeds.

\[
(D_p, \%) \text{ of 18 added fats was a linear function of } F_c \text{ (g/kg)}:
\]

\[
D_p = 76.2 + 0.094 F_c, \ r^2 = 0.45, P = 0.002.
\]

A better fit was obtained with a single exponential rising to an asymptote:

\[
D_p = 95.0 - 95.0 e^{-F_c/440}, \ r^2 = 0.50, P < 0.001.
\]

This equation estimated the asymptotic \( D_a \) of added fats and oils to be 95%. Including the basal feeds further improves the fit (Figure 1):

\[
D_p = 92.0 - 92.0 e^{-F_c/342}, \ r^2 = 0.81, P < 0.001.
\]

By analogy with the Michaelis constant of enzyme kinetics, the EE content at half maximal digestibility (46%) was 24 g/kg of feed.

An Eadie-Hofstee plot of \( D_p \) against \( D_p/F_c \) yielded a significant fit for the 18 added fats and oils (Figure 2):

\[
D_p = (102.7 \pm 5.0) - (D_p/F_c)(0.196 \pm 0.070), \ r^2 = 0.33, P = 0.013.
\]

The equation allows calculation of a maximal \( D_p \) of 103 ± 5%, and a corresponding \( F_c \) of 20 g/kg of feed at a \( D_p \) of 51.5%. Including the basal feeds gave a better fit, \( r^2 = 0.530 \), a maximal \( D_p \) of 101 ± 5%, and a EE content of 20 g/kg of feed at a \( D_a \) of 50.5%. Forcing the intercept through 100% reduced \( r^2 \) to 0.508, but adjusted \( R^2 \) remained at 0.508.

A plot of \( F_a \) against \( F_i \) reveals a linear relationship (Figure 3):

\[
F_a = F_i (1.009 \pm 0.022) - (63.01 \pm 12.23), \ r^2 = 0.990, P < 0.001.
\]

Figure 2. A plot of partial digestibilities of added fats against the quotient of digestibility and fat content in 18 feeds \( (r^2 = 0.33, P = 0.013) \). This plot is analogous to the Eadie-Hofstee plot of first-order enzyme kinetics, and it indicates a maximal or true digestibility of 102.7 ± 5.0% for added fats.

Figure 3. A plot of fat absorbed against fat intake for 23 feeds \( (r^2 = 0.99, P < 0.001) \), indicating a linear relationship \( (P < 0.001) \) with a true digestibility (slope) of 100.9 ± 2.2% and an endogenous fecal fat (intercept) of 63.0 ± 12.2 g/d.
This equation determined a $D_t$ of $101 \pm 2\%$ and an $F_{ef}$ of $63 \pm 12\, g/d$, that is, $0.22 \pm 0.04\, g\cdot d^{-1}\cdot kg\, BW^{-1}$. Forcing the regression coefficient to be 1.000 left both $r^2$ and adjusted $r^2$ at 0.990.

Hypothetical amounts of indigestible EE ($EE_n$, g/d) in basal feeds, test feeds, and added fats may be calculated assuming a $D_a$ of 100% and an $F_{ef}$ of 63 g/d. These assumptions account for the difference between $D_a$ and maximal $D_t$, when $F$ is 730 g/d, which corresponds to a $F_c$ of 163 g/kg of feed at a mean DMI of 4.47 kg/d for 23 experiments. For five basal feeds with a mean $D_a$ of 55%, the mean EE intake of 158 g/d may be partitioned into 24 g of fat with 100% $D_t$ and 134 g of $EE_n$, which would be 85% of basal EE, using the digestibility equation:

$$0.55 = [(158 + 63) - EE_n]/158.$$ 

Similarly, in 18 test feeds with a mean $D_a$ of 81%, the mean EE intake of 591 g/d may be split into 416 g of matter with 100% $D_t$ and 175 g of $EE_n$, which was 30% of test EE. Finally, for added fats with an asymptotic $D_p$ of 95%, the mean intake of 458 g/d of added fat may be divided into 372 g of matter with 100% $D_t$ and 86 g of $EE_n$, which is 19% of added fats.

**Discussion**

The results confirm previous observations of an increase in $D_a$ of EE when fats are added to forages or grains (Potter et al., 1992). The lack of associative effects of added fats on other nutrients agrees with some but not all previous reports. New findings are estimates of 100% $D_t$ of added fats in the horse and an $F_{ef}$ of 0.22 g·d⁻¹·kg·BW⁻¹. The data reveal that $F_{ef}$ failed to account for the difference between $D_a$ and $D_t$ of added fats when $F_c$ is <163 g/kg of DM. Moreover, hypothetical estimates of $EE_n$ based on $D_a$, $D_t$, and $F_{ef}$ are much larger than expected actual values of $EE_n$, so actual $EE_n$ failed to account for the difference between $D_a$ and $D_t$. The failure of these two common explanations for low $D_a$ and $D_p$ of EE up to a $F_c$ of 163 g/d suggests that some other mechanism is needed to explain low values of $D_a$ and $D_p$, especially when $F_c$ is low. A novel explanation is that enzymatic hydrolysis of fat is slow at low substrate concentrations in the small intestine as a consequence of low $F_c$ (Figs. 1 and 2). Such a mechanism would tend to increase the risk of suboptimal status of vitamins A and E in association with low-fat diets.

**Apparent and Partial Digestibilities**

The $D_a$ of 55% for the basal feeds, which contained approximately equal parts of hays and grains, fits previous estimates of 42 to 49% for hays and 55 to 76% for grains (Fonnesbeck et al., 1967; Kane et al., 1979; Sturgeon et al., 2000). The hays and grains in these previous studies had similar contents of EE, approximately 20 to 40 g/kg of DM, and the large differences between $D_a$ and $D_t$ were attributed mainly to the waxes, pigments, essential oils and other ether-extractable but poorly digested components of leaves (Van Soest, 1994).

Increased $D_a$ of EE with the addition of various fats was observed in all five trials. Similar increases have been reported in other studies of horses (Kennedy et al., 1999; Jansen et al., 2000), swine (Jones et al., 1992), and sheep (Jenkins et al., 1989). These increases in $D_a$ were attributed to dilution of indigestible components of EE in grains and especially forages by highly digestible triglycerides.

The best estimate of $D_p$ of added fats in the present study was the asymptotic value of 95% for the 18 test feeds. It may be compared with previous estimates of 88 to 94% (Bowman et al., 1979; Kane et al., 1979; Hintz and Schryver, 1989). The $D_p$ of corn oil was not greater than that of the four other fats; that is, any effects of chain length and saturation of fatty acids were not evident in the present data, which confirms previous results in horses (McCann et al., 1987). Previous results have concerned total dietary fat at less than 200 g/kg of DM, and the present results extend the upper limit to at least 230 g/kg.

**Associative Effects**

The overall lack of associative effects of added fats in the present study agrees with previous observations of DM, CP, and ADF (Bowman et al., 1979; Lane et al., 1979). Addition of fat decreases ruminal fermentation of fibers (Henderson, 1973; Perry et al., 1976; Kowalczyk et al., 1977). Signs of impaired fermentation in the equine hindgut following rapid introduction of fat are greasy and more abundant stools that may become grayish, loose, and deteriorate into frank steatorrhea (our unpublished observations). These signs are usually preceded by a sheen on well-formed fecal balls, a sign of some fat escaping digestion and an indication that the rate of fat introduction should be retarded, which takes about 4 to 14 d in healthy horses. In the present experiments, care was taken to introduce added fats gradually and to ensure acclimation. The lack of a negative associative effect on ADF is consistent with complete digestion of fat in the small intestine (i.e., a $D_t$ approaching 100%).

In contrast, a negative associative effect of soybean oil on crude fiber, NDF, and ADF has been reported (Jansen et al., 2000, 2002). This apparent adverse effect on cecal fermentation has three possible explanations. One would be a low $D_a$ specifically of soybean oil, which was present at 158 g/kg in the Dutch studies compared with only 50 g/kg in one of our feeds. Previous equine studies revealed no negative associative effect of corn oil on fiber (Bowman et al., 1979; Lane et al., 1979). Another possibility could be inadequate accommodation. However the number of days, 37 and
42 in their switchback studies (Jansen et al., 2000, 2002), should have been sufficient for accommodation, provided that introduction was gradual. A third possibility is that the $D_a$ estimate of 55% for crude fiber in the soybean oil feeds, rather than depressed, is at the maximum for typical horse feeds, and that the high $D_a$ estimate of 71% for the control feeds suggests that these may have been unusual.

**True Digestibility**

The two graphical estimates of $D_t$ of fats were both within 1.0 SE of 100% (Figures 2 and 3). Moreover, forcing the plot of $F_o$ on $F_1$ through the origin had a negligible effect on the goodness of fit, so the slope of 1.00 may be taken to represent a $D_t$ approaching 100%. We conclude that lipolysis of hydrolyzable fats is complete within the small intestine of thoroughly accommodated healthy horses. This conclusion is reinforced by the lack of negative associative effects, especially on ADF.

$D_t$ of 100% is usually assumed for fat, with $F_{ef}$ accounting for the difference between $D_t$ and $D_a$ or $D_p$. To our knowledge, the present demonstration of fat $D_t$ approaching 100% is the first in equids. A similar estimate has been found previously in other species, for example, dogs and cattle (Merritt et al., 1971; Palmquist, 1991).

**Endogenous Fecal Fat**

The present $F_{ef}$ estimate of 0.22 g·d$^{-1}$·kg BW$^{-1}$ is based on a linear regression ($P < 0.001$) of fat absorbed on fat intakes from 100 to 1,200 g/d in 23 feeds (Figure 3). A previous estimate of 0.39 g·d$^{-1}$·kg$^{-1}$ was based on a linear regression of $F_o$ on fecal DM of four diets fed to four ponies (Bryant, 1969; cited by McCann et al., 1987). Applying this empirical regression to the present data yields a mean estimate of 0.12 g·d$^{-1}$·kg$^{-1}$ for $F_{ef}$ ($P = 0.074$). An estimate of 0.13 g·d$^{-1}$·kg$^{-1}$ for $F_{ef}$ in six Jersey cows was based on a plot of fat absorbed against fatty acid intakes from 200 to 1,400 g/d (Palmquist, 1991).

**Increasing First-Order Kinetics**

The two common explanations for the difference between $D_t$ and $D_a$ or $D_p$ are insufficient to account for the present data. A calculation in the last part of the Results section shows that $F_{ef}$ accounts exactly for the difference between $D_t$ and $D_a$ when $F_o$ was 730 g/d, that is, the effect of $F_{ef}$ is excessive at $F_o > 730$ g/d and inadequate at $F_o < 730$ g/d. Another calculation indicates that the $E_{Ef}$ of the feed must be, respectively, 85, 30, or 19% of the EE in B, the test feeds, or the added fats to account for the difference between $D_t$ and $D_a$ or $D_p$. Although $E_{Ef}$ was not measured in these experiments, it is usually < 50% of the EE of forages (Van Soest, 1994), that is, much less than the 85% calculated from the balance data. Similarly, $E_{En}$ would be < 20% of the EE in B, that is, much less than the hypothetical estimate of 30%. Moreover $E_{En}$ would be negligible in the added fats, certainly much less than the estimate of 19%.

A third factor is suggested by the increasing first-order kinetics of $D_a$ and $D_p$ on $F_o$ evident in Figures 1 and 2. When $F_o$ is 20 to 24 g/kg, which is common in the staples of horse feeds (NRC, 1989), the efficiency of absorption is approximately half the maximal rate. This first-order relationship digestibility and dietary fat concentration may reflect enzyme kinetics, that is, the hydrolysis of fats by lipases (Duan, 2000). A low $F_o$ would lead to a low substrate concentration in the small intestine. A slow rate of lipolysis would be especially critical for forages, which pass rapidly from mouth to large bowel, an interval of 2 to 3 h (Holland et al., 1998). Adding fat not only increases substrate concentration but also slows gastric emptying and prolongs residence time in the small intestine (Maes et al., 1996).

Ingested fats are emulsified in the upper small intestine. Concentrations of glycerides are difficult to determine at the interface between the oil and aqueous phases (i.e., the exact site of lipase reactions). For this reason, direct information on lipase kinetics is remarkably lacking (Duan, 2000), although certain kinetic characteristics of lipases have been inferred from bile acid transport (Schiff et al., 1972). Lipid hydrolytic reactions in the small intestine are dependent not only on lipases, but also on colipase and bile salts, and may be retarded by phospholipids and proteins. Lipolytic products are removed from the oil-water interface, allowing lipolysis of remaining triglycerides to continue.

Given this situation, two assumptions are needed to sustain the hypothesis that the first order kinetics of $D_a$ and $D_p$ on $F_o$ may reflect enzyme kinetics. One is that $F_o$ and fat concentration at the interface are related. The other is that the rate of hydrolysis of fat at the oil-water interface follows typical Michaelis-Menten enzyme kinetics (Smith et al., 1983). A rate of change of substrate concentration that is dependent on substrate concentration is an example of first-order kinetics, in this case with the substrate (fat in the small intestine) decreasing and the product (fat absorbed) increasing to an asymptotic value.

The digestibility and efficiency of absorption of most nutrients increases at lower daily intakes or dietary concentrations. In contrast, it will subside at lower concentrations if it is following Michaelis-Menten kinetics. Such a response would tend to exacerbate the risk of deficiencies of essential fatty acids and fat-soluble vitamins in animals consuming low-fat diets.

The accommodation period of several days required to avoid low digestibility of added fats is consistent with substrate-adaptive lipolytic enzymes (i.e., with up regulation of lipolytic activity in response to increasing intake of fat). This regulatory mechanism may be latent in many species accustomed to diets.
containing more than 15% fat, a concentration at which fat digestibility is approaching its maximum, but overt in equids accustomed to feeds containing approximately 2% fat, a concentration at which fat digestibility is half maximum. We suspect that the increasing first-order kinetics of fat digestion described here in equids may not be a peculiarity of this species but may be found also by testing suitable ranges of fat intakes or dietary fat contents in other nonruminant mammals.

**Vitamin Status**

Low digestibility of fat at low dietary fat contents may increase the risk of suboptimal status of vitamins A and E and, perhaps, essential fatty acids. The retinol dose-response test has shown that at least twice the current recommendation for vitamin A (NRC, 1989) is required by growing ponies and pregnant Thoroughbred mares (Donoghue et al., 1981; Grieve-Crandell et al., 1993). Similarly, plasma and colostrum concentrations of retinol and α-tocopherol indicate that current recommendations for vitamin A and vitamin E are suboptimal for prepartum mares (Gay et al., 2004). These three studies used feeds with fat contents <30 g/kg of DM, and it is possible that the current recommendations (NRC, 1989) would be adequate if the efficiency of absorption were increased by fat supplementation.

**Implications**

Mean digestibilities of 55, 81, and 95% indicate mean digestible energy values of 5.2, 7.7, and 9.0 Mcal/kg of ether extract in forages, mixed feeds including added fats, and added fats, respectively, assuming a gross energy of 9.5 Mcal/kg. These caloric values should be useful in feed formulation and ration evaluation. More precise digestible energy values (Mcal/kg) may be calculated from a measured ether extract content (g/kg) using the following exponential equation: energy value = 0.095(92 – 92(ether extract/342)). Provided that added fats are introduced gradually over a period of several days, maximal ether extract digestibility is approached between 100 and 150 g/kg of dry matter and sustained to at least 230 g/kg. The dietary contents of most fats and oils, with the possible exception of soybean oil, do not exert adverse associative effects on acid detergent fiber. Decreasing digestibility at lower dietary fat contents would tend to increase the risk of suboptimal vitamin A and E status.

**Literature Cited**


