Mathematical linkage of total-tract digestion of starch and neutral detergent fiber to their fecal concentrations and the effect of site of starch digestion on extent of digestion and energetic efficiency of cattle

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ABSTRACT

Published regression equations relating digestibility of starch to fecal starch concentration have slopes that differ by over 5-fold. Hence, nutritionists have questioned their legitimacy. Total-tract starch digestibility and concentration of starch in feces are interlocked mathematically by 2 factors—starch content of the diet and digestibility of diet DM. Digestibility data compiled from the published literature including 201 diets fed to lactating dairy cows and 191 diets fed to feedlot cattle were employed to re-examine the relationships of digestibility to fecal concentrations of starch and NDF. Regression analyses and plots clearly il-

lustrated imprecision of this relationship when diet starch content and digestibility of diet DM were ignored. Furthermore, because fecal starch and diet digestibility are related inversely, mathematics implies that starch digestibility is related curvilinearly to fecal starch concentration. Effects of site of starch digestion on extent of digestion and energetic efficiency also were examined. Direct digestibility measurements for nutrients and energy can preclude errors involved with in vitro availability assays, prove more economical than laboratory procedures to predict nutrient digestibility, and provide more applicable data concerning energy availability when compared with summing tabular means for feedstuffs from publications or from computerized diet formulation programs. When combined with DMI, direct digestibility measure-

ments should markedly improve precision for quantifying amounts of nutrients and energy available for maintenance and performance by productive ruminants in dairies or feedlots. As with all analytical procedures, accurate digestibility measurement requires representative sampling and proper analysis of diets and feces.

Key words: fecal starch, starch digestibility, lactating cows, feedlot cattle, neutral detergent fiber digestibility

INTRODUCTION

The gold standard for nutrient and energy availability is direct measurement with animals under production conditions. Any starch or NDF that

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Table 1. Published equations that predict starch digestibility (SDig) from concentration of starch in fecal DM (FS)

Reference	Animal class	Regression equation: SDig =	n	R ²
Zinn et al., 2002	Feedlot cattle	$100.5 - 0.6489 \times \text{FS}$	64	0.91
Corona et al., 2005	Feedlot cattle	$104.403 - 0.715 \times \text{FS}$	16	0.97
Owens and Zinn, 2005	Feedlot cattle	$100.35 - 0.5662 \times \text{FS}$	135	0.94
Zinn et al., 2007	Feedlot cattle	$99.9 - 0.413 \times \text{FS} - 0.013 \times \text{FS}^2$	637	0.96
Owens and Zinn, 2005	Lactating cows	$98.205 - 0.9316 \times \text{FS}$	26	0.73
Lidy et al., 2009	Lactating cows	$101.92 - 2.8723 \times \text{FS}$	19	0.43
Ferguson, 2010 ¹	Lactating cows	$100.34 - 2.04 \times \text{FS}$	778	0.78
Fredin et al., 2014	Lactating cows	$100 - 1.25 \times \text{FS}$	190	0.94

¹As cited by Huibregtse et al. (2012) and Kung (2013).

has not been digested is excreted in feces, so fecal concentrations reflect indigestibility. Based on regression, numerous equations relating apparent digestibility of starch to the concentration of starch in feces have been published (Table 1). These equations in turn have been employed to predict the digestibility of starch directly from the starch concentration in fecal DM. Although fecal concentrations and digestibility of diet components are indelibly and inextricably linked, the mathematics behind this linkage reveals the presence of 2 additional factors: (a) daily fecal output that varies with indigestibility of dietary DM and (b) intake of the specific component that varies with its dietary concentration and DMI. When these 2 additional factors are known, apparent digestibility of any diet component can be calculated directly from its concentration in feces without relying on prediction equations developed through regression.

Because products of digestion and energetic efficiency can vary with site of digestion, both site and extent of digestion are important. Although site of digestion can be quantified with intestinally cannulated ruminants, numerous laboratory procedures have evolved to predict rate of ruminal digestion that, when combined with some estimate of rate of passage, provide an approximation of the extent of ruminal digestion. In vitro and in situ procedures to predict rate or extent of ruminal digestion of starch include the Degree of Starch Access

(Blasel et al., 2006), Relative Grain Quality (Hoffman et al., 2012), gas production rates, and disappearance in vitro or in situ. As outlined by Allen (2015), multiple factors alter both rate of ruminal starch digestion (e.g., exposed surface area and grain vitreousness) and ruminal residence time that varies with particle location within the rumen because of differences in floatation and density (Hooper and Welch, 1985) or evasion. These factors are difficult to simulate in vitro. For example, very high hourly rates of ruminal starch passage (16 to 26%; Taylor and Allen, 2005; Allen et al., 2008) presumably reflect ruminal segregation and preferential passage of chewed or ground dense particles potentially driven by the 18 to 80% of consumed water that sluices directly through the rumen without fully mixing and equilibrating with ruminal water (Woodford et al., 1984; Garza et al., 1990; Zorrilla-Rios et al., 1990). Such direct flushing of small dense particles is readily apparent within abomasal samples taken immediately after supplement and water are consumed.

The objectives of this paper were to examine how diet concentration and diet indigestibility alter the relationships of fecal concentrations of starch and NDF to their digestibility, to ascertain whether these 2 factors can explain why the published regression slopes and shapes differ among cattle types, to suggest additional approaches and measurements that could enhance the ease of measurement

and reliability of estimates of digestion, and to appraise effects of site of starch digestion on extent of digestion and energetic efficiency.

MATERIALS AND METHODS

Livestock were not used directly in this research. Instead, least squares means from the literature were compiled from 57 publications involving 77 trials using 201 corn-based diets fed to lactating cows, from 47 publications using 191 corn-based diets fed to feedlot cattle, and from digestion site trials conducted at the Imperial Valley Experiment Station with feedlot cattle individually fed corn-based diets. Information from publications as reported by authors was extracted regarding the starch and NDF content of the diet and digestibility of DM (or OM) and starch and NDF. Because some analytical procedures erroneously assay certain nonstarch compounds (e.g., glycogen; peptidoglycan, glycocalyx components) as starch, all digestibility values discussed below should be considered to be "apparent" starch digestibility estimates. Cell contents including starch often are presumed to have a true digestibility of 98%. However, except for steam-flaked grains and corn silage, measured total-tract apparent digestibility values for starch generally fall far below 98%.

In all regression analyses, models initially included random effects of study to account for study-to-study variability (St-Pierre, 2001) and

perhaps provide better inferences to relationships considered in the current study. Further evaluation of residuals from these models showed that scatter of data points was much less when starch digestibility values were greater. For regression estimates, weighting of individual starch digestion values by within-experiment variability typically is employed based on the SEM of the measurement. Hence, reported SEM values from the original sources were used for weighted regression analysis for the dairy data set. However, neither the distribution of residuals nor parameter estimates was altered markedly by weighting. Therefore, results from the weighted regression were not considered in this report. Instead, raw means were used throughout, matching the approach used previously by all authors who have developed equations to appraise the relationship of starch digestibility to fecal starch concentration.

Several adjustments within the data set were necessary. Three publications with lactating cows from one prestigious university in the Snowbelt reported values for DM digestibility that exceeded values for OM digestibility, an impossible condition with typical diets; the authors when contacted revealed that values for OM and DM digestibility had been switched inadvertently. In some trials, OM digestibility was reported but DM digestibility was not reported. Based on regression using data from the 149 dairy diets where digestibility of both DM and OM had been reported, the general relationship of DM digestibility to OM digestibility was determined: $\text{DM digestibility} = 1.0209 (\pm 0.016) \times \text{OM digestibility} - 3.108 (\pm 1.10)$ ($R^2 = 0.965$; $\text{CV} = 1.23$; $P < 0.0001$). For the 15 diets from trials with lactating cows where DM digestibility had not been reported and only for those diets, DM digestibility was calculated from the OM digestibility values reported for these diets using this equation. In addition, data from diets where ileal starch flow or fecal starch output exceeded duodenal starch flow were deleted based on the presumption that sampling or labora-

tory errors were responsible for these discrepancies.

The relationship of starch digestibility to fecal starch can be appraised based on either OM or DM of the diet and feces. All regression equations developed previously have expressed fecal starch as a percentage of DM. Although OM is preferable to DM as the basis for calculating diet digestibility and energetics and thereby desirable for formulating diets, digestibility of OM was reported infrequently compared with digestibility of DM within the data sets employed (149 vs. 186 of the 201 diets for dairy cows). Furthermore, assays for DM are more direct and, being simpler, are less likely to be erroneous than assays for OM.

Because starch concentration in fecal DM was reported very infrequently (20 of the 201 diets fed to lactating cows), it was calculated from other data presented in each publication. First, fecal starch output (g/100 g of dietary DM) was calculated from the starch content of the diet and the indigestibility of starch (100 minus reported starch digestibility) for each diet. Similarly, fecal output of NDF (g/100 g of dietary DM) was calculated from NDF content of the diet and the reported NDF indigestibility for each diet. Fecal DM output in grams in turn was calculated from the reported intake of DM and DM indigestibility. Finally, the percentage of starch and NDF within fecal DM was calculated for each diet based the grams of starch or NDF in feces and the total grams of fecal DM produced from every 100 g of dietary DM. Such calculations avoid the missing data and rounding errors potentially involved with tabular fecal starch concentrations. For those 20 diets where starch concentration in fecal DM had been reported, the values calculated as described above were related quite closely ($R^2 = 0.95$; root mean squared error = 0.97; $P < 0.0001$) to the tabular values reported by authors. For the individually fed feedlot cattle, starch concentration in fecal DM was available directly and therefore did not need to be calculated.

The relationships of starch digestibility to fecal starch concentration and of NDF digestibility to fecal NDF concentration were plotted (1) directly, (2) against fecal starch or fecal NDF expressed as a fraction of the dietary starch or NDF concentration, or (3) against this ratio multiplied by DM indigestibility of the diet. These relationships were based on mathematical equations known to relate fecal concentrations to digestibility. Regression lines were plotted, and graphs provided a visual appraisal of the effect of various factors on the precision with which starch and NDF digestibility could be predicted directly from these specific equations. Relationships were appraised by the relative strength of simple unweighted regressions, size of the residual SE, or correlations calculated based on all values within each data set using REG, GLM, or CORR procedures of SAS (SAS Institute Inc., Cary, NC) following the adjustments noted above. In addition, the mathematical equations known to relate fecal concentrations to digestibility were examined to determine whether these relationships were linear or curvilinear and to develop indigestibility coefficients that can be used to relate fecal concentration of any component directly to its digestibility.

RESULTS AND DISCUSSION

Individual published equations relating starch digestibility to fecal starch concentrations derived from animals within a class (Table 1) often had high correlations. In studies with feedlot cattle fed low forage diets, Zinn et al. (2007) observed that starch digestibility could be reliably predicted ($R^2 = 0.96$; $\text{Sy.x} = 0.45$; $P < 0.001$) from the starch concentration in fecal DM based on a quadratic equation. Similarly, linear equations advanced for lactating cows (Lidy et al., 2009; Ferguson as cited by Kung, 2013, and Huibregtse et al., 2012; Fredin et al., 2014) had reasonably high model R^2 (>0.78). Note that (a) the slopes of equations relating starch digestibility to fecal starch consistently were mark-

edly steeper for lactating cows than for feedlot cattle, and (b) shape of equations devised by various authors differed (curvilinear versus linear).

To further examine the relationship of starch digestibility to concentration of starch in fecal DM, data were plotted for the 201 diets fed to lactating cows as well as the 191 diets fed to feedlot cattle (Figure 1). The processing method for the corn grain that provided most of the starch within each individual diet also is denoted so readers can ascertain the relationships among individual grain processing methods.

As noted in previous regression equations of Table 1, the negative slope of the regression line relating starch digestibility to fecal starch (Figure 1) was much steeper for the diets fed to lactating cows than for the diets fed to feedlot cattle. Although model R^2 values were high within each of these 2 cattle groups, merging the data for lactating cows with those from feedlot cattle into a single regression in an attempt to develop a single universal equation reduced precision markedly: Starch digestibility, % = $98.24 - 0.55 \times$ fecal starch, % ($R^2 = 0.52$).

Slopes and intercepts of the newly calculated linear regression equations within cattle type based on unadjusted means, as used in all previous equations, generally matched those of equations published previously (Figure 2). Based on these different equations, if the measured fecal starch concentration were 10%, these 10 different equations predict that starch digestibility should average 89.2% (SD = 7.5), but these equations individually predicted starch digestibility values that ranged from 73 to 95%. This illustrates the marked disparity among these equations. Can starch digestibility be predicted accurately from measurement of the starch concentration of feces alone? Is imprecision among these estimates due to variability among animals and sampling, or is it an artifact of the relationship between starch digestibility and fecal starch concentration? Examination of the mathematical

Figure 1. Relationship of total-tract starch digestion to the concentration of starch in feces of lactating dairy cows (204 diets; solid symbols) or feedlot cattle (191 diets; open symbols) from the published literature with linear regression for each and for the combined data set. The symbol shape depicts the primary method of corn grain processing employed within each diet.

basis relating starch digestibility to fecal starch concentration can help to address these questions.

Mathematical Basis Relating Starch Digestibility to Fecal Starch Concentration

Apparent digestibility of any dietary compound as a percentage can be calculated as:

$$\text{Apparent digestibility, \%} = \frac{(\text{Intake, g} - \text{fecal excretion, g})}{\text{Intake, g} \times 100} \quad [1]$$

As applied to starch specifically,

$$\text{Apparent digestibility, \%} = \frac{(\text{Fed starch, g} - \text{Fecal starch, g})}{\text{Fed starch, g} \times 100} \quad [2]$$

For example, in trials with lactating cows, starch intake averaged 6.548 kg, whereas fecal starch averaged 532 g for an apparent starch digestibility of

91.9%. Classically, apparent digestibility is defined as true digestibility minus endogenous losses (compounds released from or secreted into but not reabsorbed from the digestive tract). Degradation and resynthesis activities by the microbial biome of the digestive tract further complicate this picture. Bacteria not only degrade starch but, particularly with a nitrogen deficiency, synthesize glycogen that is indistinguishable from undigested starch by typical assay procedures. Other compounds also can assay as starch by certain sample preparation or analytical procedures (e.g., glucose, other reducing sugars). If considered starch, such compounds contribute to dietary starch, to starch within the digestive tract, and to starch in feces (Hall et al., 2001). Method of starch analysis also can add to variation in starch digestion estimates. Among the 57 trials with lactating cows, 30 different references were cited as being the method by which starch (or starch plus glucose) was analyzed. Because method of starch analysis can

Figure 2. Total-tract starch digestion predicted from concentration of starch in feces by equations from various publications. Dashed lines are the linear regressions developed from the data sets compiled for this paper from lactating cows and feedlot cattle.

alter the measured starch content of samples (Hall, 2009), further efforts to standardize this analytical procedure are needed.

With lactating cows, Larsen et al. (2009) estimated that duodenal flow of microbial starch by lactating cows was equal to 276 g/d; daily duodenal starch flow as a component of ruminal microbes based on the starch content of isolated ruminal bacteria ranged from 360 to 1,360 g/d (Taylor and Allen, 2005), an amount equivalent to 8 to 25% of total duodenal starch flow or 5 to 18% of starch intake. Considering the small particle size of carbohydrate within ruminal microbes and the reasonably high postruminal digestibility of microbial N, digestibility of microbial glycogen and microbial starch within the small intestine would be expected to be high, although synthesis of microbial glycogen from starch might reoccur within the large intestine. The fraction of fecal starch that is present as microbial starch or glycogen with different dietary conditions or with altered digestion or passage (e.g., diarrhea) remains uncertain. The fact

that the starch concentration in feces approaches zero when starch intake approaches zero implies that the contribution of microbial starch to fecal starch is small when starch intake is low. However, the presence of non-starch compounds assaying as starch and its location (bacteria vs. protozoa) in the rumen and at the duodenum would affect ruminal energetics. Biosynthesis of glycogen by bacteria requires substantial energy input (Hall and Eastridge, 2014), whereas protozoa that simply engulf starch particles may not expend additional energy to store starch particles. The degree to which glycogen storage can alter calculated energetics of microbial growth within the rumen of cattle fed high starch diets, be altered by CP status and synchrony of protein and energy availability, and explain “energy spilling” deserves further research attention.

Rearranging Equation 2,

$$\text{Apparent starch digestibility, \%} = 100 - 100 \times (\text{Fecal starch, g/} \\ \text{Fed starch, g}). \quad [3]$$

The amount of starch consumed and excreted in feces typically is calculated from the amounts of DM fed and excreted multiplied by the starch concentrations in feed DM and in fecal DM. Expressed on this basis,

$$\begin{aligned} \text{Apparent starch digestibility, \%} = \\ 100 - 100 \times (\text{Fecal DM output, g} \times \\ \text{Fecal starch, \%}) / \\ (\text{DMI, g} \times \text{Diet Starch, \%}). \quad [4] \end{aligned}$$

So,

$$\begin{aligned} \text{Apparent starch digestibility, \%} = \\ 100 - 100 \times (\text{Fecal DM out, g/} \\ \text{DMI, g}) \times (\text{Fecal starch, \%}/ \\ \text{Diet starch, \%}). \quad [5] \end{aligned}$$

Because DM indigestibility, % = $100 \times (\text{Fecal DM out, g/DMI, g})$,

$$\begin{aligned} \text{Apparent starch digestibility, \%} = \\ 100 - (\text{DM indigestibility}) \times \\ (\text{Fecal starch, \%}/\text{Diet starch, \%}). \quad [6] \end{aligned}$$

Among the studies with lactating cows, dietary starch concentration averaged 28.12% of DM, fecal starch averaged 6.854%, and DM digestibility averaged 67.08% or, conversely, DM indigestibility averaged 32.92%, for a mean starch digestibility of 92.0%. As is apparent from Equation 6, starch digestibility automatically decreases linearly as the percentage of starch in fecal DM increases if and only if DM digestibility and starch content of the diet remain constant. Any differences in diet DM digestibility or starch concentration add a degree of complexity and imprecision to the relationship between starch digestibility and the concentration of starch in fecal DM.

Effect of Dietary Starch Concentration on Prediction of Starch Digestibility from Fecal Starch Concentration

Equation 6, being similar to previously developed general equations

for digestibility (Schneider and Flatt, 1975), clearly indicates that the concentration of dietary starch should be included in equations developed to predict starch digestibility reliably from the concentration of starch in feces. Based on the data sets summarized, starch content of 201 dairy diets averaged 28% (SD = 12%), whereas starch content of 191 feedlot diets averaged 48% (SD = 9%). Considered in light of Equation 6, this large difference in starch content between dairy and feedlot diets automatically forces the slope of the regression line relating starch digestibility to fecal starch to be much steeper for diets that contain less starch even if the other parameters remained unchanged. Furthermore, when a data set is compiled among diets where starch content is relatively consistent, the correlation between starch digestibility and fecal starch will be greater than when individual diets within a data set differ markedly in starch concentration as noted in Figure 1. Considering these published trials, the total range in starch content of diets fed to lactating cows and feedlot cattle (12 to 48% of DM and 24 to 73% of DM, respectively) was substantial. This clearly indicates that ignoring the starch content of the diet when developing a prediction equation can markedly decrease its accuracy. Likewise, for evaluating starch digestibility of a test diet, universal application of a prediction equation developed by regression will prove inaccurate if that prediction equation was derived using diets that differed markedly in starch content from that being fed in the test diet.

Expressing Fecal Starch Concentration as a Proportion of Diet Starch Concentration

When starch digestibility is plotted against the ratio of fecal to dietary starch concentration instead of fecal starch concentration alone, precision of prediction was improved for lactating cows, for feedlot cattle, and for the merged data set (Figure 3 versus Figure 1). Owens and Hassen (2011) similarly reported that total-

Figure 3. Relationship of total-tract starch digestion to the ratio of fecal starch to dietary starch concentration for lactating dairy cows (201 diets; solid symbols) or feedlot cattle (187 diets; open symbols) from the published literature with linear regression lines for each and the combined data set. The symbol shape depicts the method of processing of the corn grain employed within each diet.

tract starch digestibility was predicted more precisely when the combination of fecal starch and dietary starch was employed in the regression than when fecal starch concentration alone was employed. Yet, even after dietary starch concentration is considered, the regression equations for lactating cows versus feedlot cattle (Figure 3) remained divergent. This illustrates that one additional factor within Equation 6, diet digestibility, further modulates the relationship of starch digestibility to concentration of starch in feces.

Effect of Diet Digestibility on Predictability of Starch Digestibility from Concentration of Starch in Feces

As noted in Equation 6, the relationship of starch digestibility to fecal starch depends on the indigestibility of dietary DM. Because forages and most by-product feeds usually are less digestible than grains, including a higher percentage of such materials in

a diet generally decreases digestibility of diet DM, e.g., adding indigestible DM to the diet increases fecal DM output and dilutes the starch concentration of feces. The mean DM digestibility from these published trials with lactating cows was 67% (SD = 5%), but its range again was large (49 to 79%). This range can alter the regression coefficient relating starch digestibility to concentration of starch in feces markedly. Among the published trials with feedlot cattle, mean DM digestibility was 78.7% (SD = 5.0%), but its range also was surprisingly broad, 59.5 to 91.5%. To adjust for both dietary starch and DM indigestibility, one can plot starch digestibility against fecal starch divided by dietary starch multiplied by diet indigestibility. When expressed in this fashion, total-tract starch digestibility was determined precisely (root mean squared error = 0; $R^2 = 1.00$; graph not shown) for both lactating cows and feedlot cattle as would be expected from Equation 6. Consequently, these experimental results clearly sup-

port the contention that digestibility of starch mathematically represents the combined effects of the concentrations of (a) starch in fecal DM, (b) starch in diet DM, and (c) the indigestibility of DM. Thus, including some estimate of diet digestibility should improve the accuracy for calculating starch digestibility from the combination of starch concentration of the diet and of fecal DM. Likewise, Zinn et al. (2007), considering the curvilinearity of the relationship between starch digestion and fecal starch concentration, observed that the precision for estimating starch digestion from fecal starch concentration was enhanced by incorporating one index of DM indigestibility, i.e., fecal N, into the prediction equation when the concentration of starch in fecal DM exceeded 5%. Accuracy of starch digestibility estimation relies on representative sampling and proper analysis for feed and fecal samples for components of interest as well as the accuracy of digestibility measurements determined by total collection or marker procedures.

Confirmation Based on Trials with Individually Fed Feedlot Cattle

The relationship of starch digestibility to fecal starch for individually fed feedlot cattle receiving diets containing corn grain processed by various methods is presented in Figure 4. Both the linear and curvilinear components of the equation were significant ($P < 0.0001$). More extensive grain processing (e.g., flaking) generally resulted in lower fecal starch concentrations and higher starch digestibility, an effect that parallels the typical response to grain processing noted within other data sets (Owens and Zinn, 2005; Figure 1). But as discussed by Zinn et al. (2011), within each processing method, additional factors alter starch digestibility, e.g., density of flaked grains, particle size and density of the individual particles generated from grains by processing, moisture content and duration of storage for fermented grain or silage,

Figure 4. Relationship of total-tract starch digestion to fecal starch concentration by 511 individually fed feedlot cattle. Symbols depict the method of corn grain processing. Both the linear effect ($-0.362 + 0.014$) and the quadratic effect ($-0.012 + 0.000492$) components of the equation were significant ($P < 0.0001$).

and dilution by other starch sources in the diet. When starch digestibility from this data set based on individual cattle was plotted against fecal starch divided by starch content of each diet multiplied by diet DM indigestibility, a perfect inverse relationship (root mean squared error = 0; $R^2 = 1.00$; graph not shown) was obtained that was similar to that noted previously from digestion trials based on diet means for lactating dairy cows and feedlot diets.

Linearity Versus Curvilinearity of Regression Equations

Equation 6 appears linear. What might explain the quadratic relationship ($P < 0.0001$) between starch digestibility and fecal starch that was apparent within the regression equation derived by Zinn et al. (2007) and in Figure 4?

The fact that starch digestibility can be determined directly from fecal starch following consideration of both dietary starch concentration and diet

digestibility should not be interpreted to mean that this relationship is linear if the individual components within Equation 6 interact. Equation 6 includes 2 measures related to digestibility—starch concentration of feces, and DM digestibility of the total diet. These 2 factors are not independent. If the digestibility of the nonstarch components remains unchanged, any increase in the fecal starch concentration automatically causes DM digestibility to decrease. As a result, within a diet and animal, fecal starch and diet digestibility will change simultaneously but in opposite directions so that starch digestibility decreases at an increasing rate as fecal starch increases. This in turn forces the relationship of starch digestibility to fecal starch to be curvilinear as noted by Zinn et al. (2007) and Figure 4. To what degree are digestibility of DM and digestibility of starch related if starch intake remains constant? Regressions were calculated for the higher forage diets fed to lactating cows and the higher

grain feedlot diets within individual trials where specific diets had similar compositions and starch content but starch digestibility differed. The following relationships were detected:

Higher forage diets:

$$\begin{aligned} \text{DM digestibility, \%} &= 36.01 + 0.337 \\ &\times \text{apparent starch digestibility, \%}; \\ n &= 77; R^2 = 0.91; P < 0.01. \end{aligned}$$

Higher starch diets:

$$\begin{aligned} \text{DM digestibility, \%} &= 18.17 + 0.598 \\ &\times \text{apparent starch digestibility, \%}; \\ n &= 10; R^2 = 0.32; P < 0.01. \end{aligned}$$

The R^2 value with higher starch diets was lower presumably because the range in starch digestibility was less for the higher starch than the higher forage diets. Nevertheless, within each diet type, the digestibility of DM increased ($P < 0.01$) as starch digestibility increased. These specific forage and concentrate diets had mean starch contents of 28 and 51%, respectively. When compared with the regression slopes detected, the increases in DM digestibility calculated by regression exceeded (being 120 and 117%; $0.337/0.28$; $0.598/0.51$) those expected from starch digestibility changes alone. This implies that the digestibility of some diet component(s) beyond starch (e.g., nonstarch components of corn grain, other diet ingredients) also must have increased as starch digestibility increased. Starch typically comprises 72% of corn grain DM. Consequently, a substantial increase in the digestibility of the nonstarch components of corn grain (28% of corn grain DM) could fully account for this additional increase in diet digestibility as proposed previously by Zinn et al. (2002). Grain processing or treatments that affect particle oversize or disrupt the pericarp to decrease steric or hydrophobic hindrance of digestion would increase the accessibility of both starch and nonstarch components of grains for attack by

Figure 5. Projected relationship of total-tract starch digestion to fecal starch assuming that low (28%) starch diets had a DM digestibility of $36.01 + 0.337 \times$ starch digestibility and that higher (48%) starch diets had DM digestibility of $18.17 + 0.598 \times$ starch digestibility.

microbial or animal enzymes. Hence, an increased digestibility of nonstarch components potentially can explain the larger change in DM digestibility than is available from starch alone. Note, however, the fact that DM digestibility increases simultaneously with starch digestibility should not be construed to indicate that DM digestibility will increase as starch content of a diet increases. The concentration of starch in the diet can alter other factors that affect digestion, e.g., pH and microbial activity within various segments of the digestive tract, residence time for digestion, and ruminal fill. These changes in turn may have negative associative effects on digestibility of various feed components. That the production response by lactating cows to dietary starch concentration also varies with level of milk production was clearly illustrated by Boerman et al. (2015).

To what degree does an alteration in starch digestibility and thereby in DM digestibility alter the degree of curvature in the relationship of starch digestibility to fecal starch? Inserting

values for the effect of starch digestion on DM digestion into Equation 6 based on mean values for starch contents of diets with higher forage or higher starch content (28 and 51%, respectively) yielded the curves shown in Figure 5. Note that as expected the deviation from linearity was considerably greater for diets with greater starch content. Failure of many previous equations to detect this curvilinearity presumably reflects an imprecision among starch digestibility measurements within the data sets that were employed to generate these regression equations.

Does curvilinearity of the relationship of starch digestibility to fecal starch concentration apparent in Figure 4 alter the validity of Equation 6? Within a diet and animal, differences in DM digestibility automatically are considered when DM digestibility is included as an independent factor in Equation 6. Thus Equation 6 remains valid regardless of any positive or negative effects of starch on digestibility of other diet components because DM digestibility already is an

inherent component of the equation. In contrast, for individual animals fed the same diet, the relationship of digestibility to fecal starch concentration will be curvilinear because as fecal starch concentration increases, DM digestibility typically decreases.

NDF Digestibility Versus Fecal NDF

Dairy nutritionists have expressed disappointment that, unlike for starch, NDF digestibility by lactating cows cannot be immediately and reliably predicted directly from the NDF content of fecal DM (Cotanch, 2011). Sophisticated in vitro procedures to predict total-tract NDF digestibility that employ multiple in vitro measurements [undigested NDF (uNDF), potentially digested NDF] and rates of potentially digested NDF digestion and passage have been developed and tested (Lopes et al., 2015). Measured in vivo NDF digestibility was matched more closely ($r = 0.49$) by multifactor in vitro methods than by in vitro measurements of NDF digestion at a single time point (Rock River Lab, 2015). Though in vitro prediction equations ideally should exhibit strong correlations as well as numeric equivalence with in vivo measurements, not merely against other in vitro procedures, few in vitro procedures have been compared directly with in vivo measurements. Using the data set for lactating cows, the relationship of NDF digestibility to NDF concentration in feces was plotted (Figure 6).

As with starch, re-expressing fecal NDF as a proportion of NDF content of the diet strengthened the relationship (Figure 7) even though precision remained low for predicting digestibility of dietary NDF due to the wide range in diet DM digestibility. Within the compiled data sets, the CV was greater for dietary NDF concentration than for DM digestibility both with dairy (13.5 vs. 7.1%) and feedlot diets (29.1 vs. 6.1%). Expressing fecal NDF as a fraction of dietary NDF multiplied by diet DM indigestibility fully accounted for all differences in NDF digestibility (root mean squared error

Figure 6. Relationship of total-tract NDF digestion to fecal NDF concentration by lactating dairy cows (201 diets) from the published literature. Symbols indicate the primary source of dietary NDF.

= 0; $R^2 = 1.00$; graph not shown). This indicates that following consideration of both the dietary concentration and indigestibility of DM,

as discussed earlier for starch, NDF digestibility was predicted accurately and precisely from the NDF concentration in feces.

Figure 7. Relationship of total-tract NDF digestion to the ratio between fecal NDF and dietary NDF concentration by lactating dairy cattle (201 diets).

Field Application

Reliable digestibility estimates are dependent on obtaining samples that are representative of the diet consumed and of fecal material. When diet sorting occurs, obtaining a representative sample of the diet consumed by an individual animal in a group or by a group of animals becomes very difficult. The effect of sorting on digestibility estimates presumably can be counterbalanced most readily by combining fecal samples from a large number of cattle within a group or by assaying individual samples from multiple animals within a group. Methods to obtain a representative fecal sample from multiple animals have been discussed by various workers including Sartec (2007) and Rock River Lab (2015). Fortunately, calibrated near infrared analysis for starch content of feces allows rapid and economic analysis of samples from individual animals within a group. Considering that fecal material has fermented for 12 h in the large intestine before defecation, sampling freshly voided undisturbed feces rather than obtaining samples directly from the rectum should be sufficient and circumvent the need for the "Little Jack Horner" approach to fecal sampling. A slight but gradual loss of starch during sample storage simulating transport to an analytical laboratory was reported by Haerr et al. (2014), so refrigeration of samples before analysis is recommended. (This could justify purchase of a separate refrigerator to store appropriate samples as well as imbibable liquids.) The degree that freezing or microwave drying might alter assayed starch values needs further study. Through analysis of representative samples of the diet (TMR) and feces as well as a digestion marker, the digestibility of diet DM, starch, NDF, ADF, and nonstarch non-NDF DM (by difference) could be calculated once matched samples are assayed for the appropriate components. Conveniently, uNDF120 has been employed as an indigestible marker in some digestion trials and field studies (Schalla et al.,

2012). Unfortunately, the concentration of ADL within uNDF240 present in the diet of 26 different dairy herds was related only weakly ($R^2 = 0.24$) to its concentration within fecal uNDF240 (Powel-Smith et al., 2015). Based on its historical reliability, lignin assayed by some appropriate and repeatable method may be preferable to indigestible NDF as an inherent digestibility marker although other verified inherent or added digestion markers (Owens and Hanson, 1992) should prove suitable.

Commercial Value of Starch Digestibility Estimates

How an alteration in starch digestibility will affect animal performance (e.g., daily milk yield; ADG) is difficult to predict. The potential degree that production or performance could increase can be calculated from the amount of additional energy available from a given quantity of diet. However, an increase in the supply of ME can result in a decrease in DMI, so ultimately an animal's response can occur either in (1) rate of production, (2) efficiency of production (energy corrected milk/DMI or G:F), or (3) both. When DMI is regulated by chemostatic factors rather than ruminal fill, an increase in energy availability will decrease DMI; consequently, ADG or milk yield will not increase as dietary energy content is increased, although efficiency of production per unit of feed should increase. Compared with small changes in production rate, small changes in efficiency are detected less readily by livestock producers. Also, because production responses can vary with numerous factors, quantitatively predicting a response in daily milk yield or ADG from a given increase in energy availability can prove misleading or erroneous. Yet, based on comparisons among starch digestibility means from 7 different corn processing methods summarized by Firkins et al. (2001), daily milk production increased by an average of 0.20 kg or 0.6% for each 1% increase in total-tract starch digestibility ($R^2 = 0.90$; $P < 0.01$).

Likewise, they reported that the milk yield:DMI ratio increased by 0.006 units (0.4%; $R^2 = 0.32$; $P = 0.19$) for each 1% increase in total-tract starch digestibility.

Effect of Site and Extent of Digestion on Energetic Efficiency

Extensive grain processing (flaking, fermentation) not only increases total-tract digestibility of starch but also typically shifts site of starch digestion back toward the rumen and reduces the amount of starch flowing to the duodenum (Zinn et al., 2002). Energetic efficiency with which digested or fermented starch is used by ruminants varies with site of starch digestion, being lower for starch fermented to VFA in the rumen than for starch digested to glucose that presumably could be absorbed from the small intestine. As determined by infusion studies with glucose or starch, by G:F regression, and calculations based on methane and heat losses associated with ruminal digestion, the estimated energy recovery from starch that is digested in the rumen has ranged from 69 to 86% of that derived from starch digested in the small intestine (Owens et al., 1986). Calorimetric studies by McLeod et al. (2001) indicated that energy recovery from ruminally fermented starch was 74% that of starch digested in the small intestine. However, as clearly illustrated by Huntington et al. (2006), starch reaching the small intestine is not fully digested therein; any residual undigested starch reaches the large intestine where it again may be partially fermented but with an energetic efficiency even lower than for starch fermented within the rumen. Based on relative efficiencies of energy use of 80, 97, and 62% for starch digested in the rumen, small intestine, and large intestine as estimated by Huntington et al. (2006), the point at which energetic efficiency of use of energy would be increased by an increase in ruminal starch escape will vary with extent of starch digestion in the small plus large intestine. If digestion of

starch in the large intestine were zero, starch digestion in the small intestine would need to exceed 82% ($80/0.97$) to counteract the lower energetic efficiency of ruminal digestion. However, starch fermentation in the large intestine compensates partially for incomplete starch digestion in the small intestine. Considering the extent of starch digestion in the large intestine and its efficiency of use, the small intestinal starch digestibility percentage above which efficiency of energy use would be improved by an increase in ruminal starch escape follows this formula: Duodenal starch digestion, % = $(80 - 0.62 \times \text{large intestinal starch digestion, \%}) / (0.97 - 0.0062 \times \text{large intestinal starch digestion, \%})$. When starch digestion in the large intestine is zero, the break point occurs at 82% as expected. But if starch digestion in the large intestine were 100%, this break point occurs when duodenal starch digestion exceeds 51% ($18/0.35$). Considering that the mean extent of starch digestion in the large intestine among corn grain processing methods ranges from 17 to 74%, the minimum small intestinal starch digestibility needed for an increase in small intestinal to be advantageous energetically will range from 66 to 80%, depending on the type and extent of grain processing.

Data from the 38 diets with cattle where starch digestion in the rumen and small and large intestine each had been measured were subdivided by method of corn grain processing. As a percentage of starch intake, starch disappearance in the rumen was greater ($P < 0.05$) with flaked than with dry rolled corn and for dry rolled than for ground diets (85 vs. 79 and 67%, respectively). In addition, extent of starch digestion in both the small and large intestine was greater when diets were based on corn grain that was ground or steam flaked than when the grain was dry rolled; means for small intestinal apparent digestibility of starch reaching the duodenum were 76, 82, and 55%, whereas apparent digestibility in the large intestine as a fraction of starch entering was 74, 64, and 17% for flaked,

ground, and rolled corn, respectively (data not shown). Calculated efficiencies with which digested starch would be used when digested at various sites based on the coefficients advanced by Huntington et al. (2006) are presented in Figure 8. Note that all energy efficiency values fell remarkably close to the standard ratio of ME to DE of 82% for beef cattle (NRC, 1996). Only when grain was steam flaked was efficiency of energy use significantly but negatively related to extent of ruminal digestion. By regression, energetic efficiency appeared to increase by 0.20 percentage points for each 1% decrease in ruminal starch digestion. This indicates that decreasing the extent of ruminal starch digestion with a steam-flaked corn diet from 90 to 75% should increase energetic efficiency of use of digested starch by 3.3%. This supports the concept that energetic efficiency can be increased slightly when site of starch is shifted postruminally if starch digestibility in the small intestine is sufficiently high. Increasing duodenal starch flow did not and should not prove consistently beneficial energetically with corn grain that

was dry rolled because of the lower small intestinal starch digestibility for dry rolled corn grain. Postruminal starch digestibility typically is high with small grains (oats, barley), with flaked corn, and with fermented corn grain or the grain within corn silage where small intestinal starch digestibility by lactating cows for corn silage stored for 5 mo was high (78 to 91%; Jensen et al., 2005). Total-tract starch digestibility of processed corn silage remained high even with more mature corn silage (up to 40% DM) based on meta-analysis (Ferraretto and Shaver, 2012). With diets containing starch from these sources, altering feeding conditions to increase ruminal starch escape (e.g., added dry forage to increase saliva flow; high feed intakes; meal feeding of concentrate) should slightly improve the net energy value of digested starch.

The practical importance of diet formulation or grain processing to alter site and extent of digestion must consider not only effects of processing on energetic efficiency of use of starch that is digested, but also the effect of processing on extent of total-tract starch digestion. Total-tract starch

Figure 8. Theoretical efficiency of converting energy from digested starch into retained energy by steers fed corn grain processed by various methods.

digestion was significantly greater for corn that had been either steam flaked or finely ground than for corn that had been rolled, averaging 99.0, 98.1, and 93.7%, respectively. Note that this ranking differs from the rank order reported by Corona et al. (2005) where processing methods were compared within a single study reflecting the fact that when combined across trials, differences in analytical and processing procedures may cause results to differ. Nevertheless, as a result of these differences in total-tract starch digestibility across trials, efficiency with which energy from dietary starch should have been used by cattle in these studies was significantly greater for flaked and finely ground corn than for rolled corn (81.1 and 81.1 versus 75.4%) as illustrated in Figure 9. Note that the effect of processing method on energy availability of dietary starch was driven to a much greater extent (partial regression coefficients ranging from 0.83 to 0.95) by differences in total-tract starch digestibility than by differences in the efficiency of use of digested starch. As outlined by Taylor and Allen (2005), numerous additional factors can be altered either beneficially or detrimentally by site of starch digestion (e.g., yield of microbial protein, ruminal pH with its effect on NDF digestion, potential for acidosis, DMI) and must be considered in addition to diet and grain processing cost when diets are being formulated or grain is being processed to alter site and extent of starch digestion.

Recovery in the portal blood stream of the glucose that disappears from the small intestine is far from complete as reviewed by Harmon et al. (2004) and Harmon and Taylor (2005). This has raised a concern about the fate or destiny of energy from glucose that disappears within the small intestine. Observed increases in the respiratory quotient with abomasal glucose infusion and measured increases in the mass of omental and internal fat reserves indicate that much of the glucose absorbed from the small intestine is used for lipogenesis within the viscera. This limits its

value as a source of blood glucose for metabolism or lactose synthesis. Visceral synthesis of lipid also conflicts with the general concept that lipid synthesis by ruminants occurs primarily if not exclusively within peripheral adipose and mammary tissues generally being derived from acetate and butyrate. Despite widespread concerns about the adverse effect of visceral fat accumulation on human health, visceral fat synthesis and retention has received surprisingly little research attention in any other species. Factors such as cattle breed and energy status also may influence the site and extent of lipid accumulation versus its mobilization and transport to other sites for deposition, secretion, or catabolism. The degree that substrate for and location of fat synthesis might alter the composition of depot or milk fat or the ease of disposal of excess heat generated during lipogenesis deserves further research attention.

The amount of fat that potentially could be synthesized daily within the viscera if all of the disappearing glucose were used for fat synthesis can be estimated from small intestinal starch disappearance and its potential for fat

synthesis. Flatt (1970) indicated that synthesis of one mole of tripalmitin required 15 moles of glucose, a mass equivalent of 33% of glucose being converted to triglyceride. Based on this estimate, daily yield of fat potentially synthesized by the small intestinal viscera was computed from the amount of glucose disappearing from the small intestine. To place such values in context, they were compared with daily fat accretion rates calculated for growing-finishing steers based on typical rates of gain, net energy equations, and daily DE intake with each diet. Daily fat synthesis by the viscera calculated from small intestinal glucose disappearance was equal to over 70% of daily fat accretion by rapidly growing steers fed ground corn grain diets. With flaked or dry rolled corn, synthesis of fat from glucose by the viscera could account for between 30 and 40% of daily fat accretion for growing steers fed flaked and dry rolled corn. The fraction of daily milk fat secreted that could be attributed to synthesis from glucose absorbed from the small intestine in one study with rolled or ground dry corn ranged from 42 to 61% (Remond

Figure 9. Theoretical efficiency of converting energy from consumed starch into retained energy by steers fed corn grain processed by various methods.

et al., 2004), but in another study when rolled or high moisture corn was fed, it ranged from only 0 to 20% (Knowlton et al., 1998). These higher rates of fat synthesis daily within the viscera when compared with daily empty body fat accretion or milk fat secretion reveals that synthesized lipid cannot continue accumulating within the viscera but instead must be transported to other sites for deposition, secretion, or metabolism.

Limits to Postruminal Digestion of Starch

Is there a ceiling to the amount of starch that can be digested within the small intestine? If so, the quantity of postruminal starch disappearing from the small intestine or total digestive tract should plateau when duodenal starch flow exceeds some quantity. Because ileal starch flow measurements are rare, the response in postruminal starch digestion (small plus large intestines) was used as a proxy, albeit incomplete, for small intestinal starch digestion. Effects of duodenal starch supply on postruminal starch digestion with both dairy and feedlot diets were examined within each grain processing method. Based on feedlot diets, postruminal starch digestion increased linearly within each corn grain processing method at least to 2,200 g daily (Figure 10). Similarly, with dairy diets, except for those diets supplemented with rolled corn, postruminal starch digestion increased consistently as duodenal starch flow increased to at least 4,900 g daily (Figure 11). No plateau or ceiling in the quantity of starch disappearing postruminally was evident within any of these grain processing methods. However, postruminal digestibility of duodenal starch (e.g., the slope) differed among these grain processing methods, paralleling the differences among grain processing methods in total-tract starch digestibility.

When expressed as a percentage of duodenal starch, postruminal starch digestion of duodenal starch by feedlot cattle fed whole or steam-flaked corn diets remained largely

Figure 10. Daily postruminal starch digestion versus daily duodenal starch flow (g/d) for feedlot cattle being fed corn grain processed by various methods.

unchanged by the quantity of duodenal starch. With high moisture corn, postruminal starch digestion tended to increase as duodenal starch supply

increased, although this relationship is driven largely by a very low postruminal digestibility from 1 of the 6 high moisture corn diets (Figure 12).

Figure 11. Daily postruminal starch digestion versus daily duodenal starch flow (g/d) for lactating dairy cows being fed corn grain processed by various methods.

With rolled corn diets, the scatter in postruminal starch digestibility values was very large. With dairy diets, postruminal starch digestibility tended to remain unchanged by duodenal starch supply with flaked and with high moisture corn. As duodenal starch supply increased, postruminal starch digestibility tended to increase with ground corn but to decrease with rolled corn diets (Figure 13), presumably due to differences in particle size and vitreousness among these processed grains. Ramos et al. (2009) demonstrated that particle size reduction increased in situ digestibility of starch both in the rumen and postruminally and that adverse effects of highly vitreous flint corn on starch digestibility were obliterated by fine grinding of maize grain.

Does altering the extent of ruminal digestion alter total-tract starch digestibility? These comparisons for feedlot cattle and for lactating dairy cows are shown in Figures 14 and 15, respectively. With feedlot diets, increases in the extent of ruminal starch digestion with rolled or whole corn diets tended to be associated with increases in total-tract starch digestion, whereas for flaked grain and high moisture corn, extent of ruminal starch digestion had little impact on extent of total-tract starch digestion. In sharp contrast, with lactating cows more extensive ruminal starch digestion tended to be associated with an increased extent of total-tract starch digestion with flaked and high moisture corn diets. But with rolled or ground corn, duodenal starch supply had limited impact on total-tract starch digestion of starch from corn grain. Differences with processing method presumably reflect alterations in site of total-tract starch digestion such that with certain processing methods and diet types, postruminal starch digestion largely compensated for incomplete ruminal starch digestion. The difference between feedlot and dairy diets in response to grain processing method likely represents a combination of or interactions among various factors, e.g., more extensive mastication and particle size reduction by

Figure 12. Postruminal starch digestibility by feedlot cattle fed diets yielding different amounts of duodenal starch daily being fed diets containing corn grain processed by various methods.

feedlot cattle than by lactating cows, shorter retention time for digestion of grain particles in the rumen with the higher intakes and higher fiber concentrations of dairy diets, and shorter

retention time in the large intestine with higher fiber diets that limits the time for compensatory digestion of starch that has resisted digestion in the rumen and small intestine.

Figure 13. Postruminal starch digestibility by lactating dairy cows fed diets yielding different amounts of duodenal starch daily being fed diets containing corn grain processed by various methods.

Is It Preferable to Model or to Measure?

Numerous models have been designed to predict livestock performance from feed intake plus historical estimates of energy and nutrient availability or of digestibility often measured at a maintenance level of intake. Occasionally, certain *in vitro* chemical analysis of the specific TMR being fed or its ingredients are included within a prediction model. Balancing diets based on input alone while ignoring multiple outputs is similar to developing a budget based on income alone while ignoring expenses. Direct measurements of energy and nutrient digestibility based on feed and fecal analysis using home-grown and home-processed feed ingredients being fed to high producing cattle should provide appraisals of energy and nutrient availability that are more applicable locally than generalized models can provide (Schalla et al., 2012). Ideally, future models should allow incorporation of field measurements of digestibility to modulate specific prediction equations and provide checks and balances that can be used to improve model reliability and applicability. Meanwhile, fecal starch measurements can readily evaluate and quantify the usefulness of and potential benefits from adequate processing of grain and corn silage (Fredin, 2014) and appraise the value of home-grown forages or grains that may be fed for a full year. Unfortunately, total-tract digestion estimates do not provide information that can be used to predict site of digestion, a factor of interest for estimating microbial protein yield, post-ruminal protein supply, and energetic efficiency as discussed above.

Analysis of a feed or a TMR plus a fecal sample for starch, NDF, plus lignin at several feed analysis laboratories currently costs less than analysis of a single sample (e.g., TMR) for NDF disappearance at 24 h plus *in vitro* starch digestion at 7 h. Reliability and applicability of digestibility measurements should be greater for *in vivo* than for *in vitro* estimates considering that *in vitro* assays are

Figure 14. Total-tract starch digestibility versus ruminal starch digestion for feedlot cattle being fed diets containing corn grain processed by various methods.

affected by numerous and interacting extraneous factors (e.g., sample drying method, kernel maturity, size and density of particles generated by sample grinding, fermentation time, sifting through *in situ* bags, and evaluation of ruminal digestion) as outlined by Philippeau and Michalet-Doreau (1997) and Allen (2015). As indicated by Zinn et al. (2007), the NE values for dietary grains and the NE responses to grain processing were

affected by numerous and interacting extraneous factors (e.g., sample drying method, kernel maturity, size and density of particles generated by sample grinding, fermentation time, sifting through *in situ* bags, and evaluation of ruminal digestion) as outlined by Philippeau and Michalet-Doreau (1997) and Allen (2015). As indicated by Zinn et al. (2007), the NE values for dietary grains and the NE responses to grain processing were

Figure 15. Total-tract starch digestibility versus ruminal starch digestion for lactating dairy cattle being fed diets containing corn grain processed by various methods.

correlated quite closely with total-tract starch digestibility for cattle fed starch-rich feedlot diets. This indicates that in vivo digestibility of starch plus NDF could be employed directly to calculate NE values for diets under production conditions via energy availability equations developed by the NRC (1996, 2001) for feedstuffs.

Although direct measurements of total-tract digestibility should prove more reliable and precise than values predicted from in vitro methods, laboratory methods remain a necessary adjunct to animal-based measurement in several arenas. First, for plant breeders, a very large number of cultivars or hybrids must be screened rapidly using small samples. Second, for novel or variable feed ingredients, laboratory methods can provide comparative values rapidly for diet formulation and pricing. Third, for appraising nutritive value of forages, in vitro procedures currently form an important basis for trade and pricing. Fourth, the comparative economic benefit from and the standardization of processing methods for forages and grains can be rapidly assessed through appropriate laboratory procedures. Finally, to estimate the potential extent of digestion of feed ingredients and quantify the rate of digestion of potentially digestible fiber, long-term in vitro or in situ incubation methods and gas evolution procedures provide essential benchmark values. Unfortunately, many in vitro analytical procedures have been designed to simulate the presumed time for digestion, often without considering rate of passage and differential passage. And, some laboratory methods have been optimized simply to reduce the variability of results, to increase repeatability of results within or across laboratories, or to match time schedules of laboratory personnel. Many in vitro procedures have been “verified” only through their correlation with some other unverified in vitro procedures, not against the gold standard of animal-based measurements of site and extent of digestion of energy and of specific feed components. To avoid

Table 2. Effect of dietary starch concentration and digestibility of dietary DM on the multiple of fecal starch (*Ki*) that equals starch indigestibility

Dietary starch, %	Diet DM digestibility, %				
	60	65	70	75	80
15	2.67	2.33	2.00	1.67	1.33
25	1.60	1.40	1.20	1.00	0.80
35	1.14	1.00	0.86	0.71	0.57
45	0.89	0.78	0.67	0.56	0.44
55	0.73	0.64	0.55	0.45	0.36

misleading readers of scientific publications, digestibility values reported in scientific publications that have not been determined with animals should be preceded by the most appropriate qualifying term, e.g., in vitro, in situ, in sacco, or in silico. Observed responses based only on laboratory estimates or computer predictions of digestibility should be considered tentative until animal measurements either confirm or refute such differences.

Calculating Digestibility from Analysis of Feces

Starch Digestibility. The rate at which starch digestibility decreases as fecal starch concentration increases can be calculated directly from Equation 6 by rearranging terms as shown below:

$$\begin{aligned} \text{Apparent starch digestibility, \%} = \\ 100 - (\text{DM indigestibility} / \\ \text{Diet starch, \%}) \\ \times (\text{Fecal starch, \%}) \times 100. \end{aligned} \quad [7]$$

This equation indicates that the rate at which starch digestibility declines for each 1% starch in fecal DM, defined herein as *Ki*, mathematically can be defined as

$$Ki \text{ for starch} = \text{DM indigestibility, \%} / \text{Diet starch, \%}.$$

Solving for *Ki* with diets covering a broad range in dietary starch concen-

trations and DM digestibility values yields the general matrix shown in Table 2.

This matrix indicates that for a lactating cow fed a diet containing 25% starch with a DM digestibility of 70%, starch digestibility declines by 1.2 percentage points for every 1% starch in fecal DM. The difference between this value and slopes noted in Table 1 for dairy diets (0.93 to 2.87) can be ascribed partly to deviations from the true intercept (that mathematically must be 100% when fecal starch is zero) and partly to differences in the starch concentration and DM digestibility among diets. By comparison, for feedlot cattle fed a diet containing 45% dietary starch with a DM digestibility of 80%, starch digestibility declines by 0.44 percentage points for each 1% starch in fecal DM. Again, differences among intercepts of various equations and variability in the dietary starch content and DM digestibility of diets included in the data sets used for equation development can explain the spread among slopes noted in Table 1 for feedlot diets (0.44 to 0.71).

NDF Digestibility. In a fashion similar to that used for starch above, *Ki* values for NDF can be generated to calculate the rate of depression in digestibility per unit of NDF in feces:

$$Ki \text{ for NDF} = \text{DM indigestibility, \%} / \text{Diet NDF, \%}. \quad [8]$$

Solving for the *Ki* of NDF for diets covering the range in dietary NDF

concentrations and DM digestibility values for lactating cows and feedlot cattle yields precisely the same matrix as shown for starch in Table 2 even though the inputs differ (dietary NDF replacing dietary starch). As a result, the K_i value for NDF would be identical to the K_i for starch (Table 2) only when dietary nutrient concentrations are identical. Because K_i is derived from the dietary concentration of a specific nutrient or compound, any K_i value must be considered unique to that specific nutrient or compound.

The matrix in Table 2 indicates that for a lactating cow fed a diet containing 30% NDF with a DM digestibility of 70%, NDF digestibility declines by 1.0 unit for each 1% NDF in fecal DM. If fecal NDF was 45% of diet DM and K_i was 1.0, NDF digestibility would be 55% ($100 - 45 \times 1$). For feedlot cattle fed a diet containing 15% dietary NDF with a DM digestibility of 80%, NDF digestibility declines by 1.33 percentage points for each 1% NDF in fecal DM. So if fecal NDF were 25% of fecal DM, NDF digestibility would be 68% ($100 - 25 \times 1.33$). To examine effects on apparent total-tract digestibility of

nonstarch cell contents of the diet, the sum of NDF and starch can be subtracted from the diet DM to generate K_i values for the nonstarch non-NDF fraction of the diet.

The effects of altering the dietary concentration of a component and the digestibility of the diet on the K_i value for any component are illustrated in Figure 16; values ranged from 0.36 (based on a diet with an 80% DM digestibility when the diet component of interest comprised 55% of diet DM) to 8.0 (based on a diet with a DM digestibility of 60% with only 5% of the diet being the component of interest). Whenever digestibility of DM or concentration in the diet decreases, K_i increases. Note that K_i is specific for a diet component (e.g., starch, NDF) and cannot be generalized across diet components.

Interpreting Results from Feed Analysis Laboratories

Because the decline in starch or NDF digestibility for each percentage point of starch or NDF in feces differs with dietary starch or NDF concentrations and DM digestibility,

diligent commercial analytical laboratories that assay fecal starch or NDF concentrations should provide clients not only with fecal concentrations but also with K_i values or Table 2 plus a suggested zone of confidence for the derived estimate rather than calculating apparent digestibility from fecal concentration based on a single published regression equation. On sample submission forms, analytical laboratories could request clients or consultants to provide estimates of the concentration of starch or NDF in the specific diet being fed to the animals that donated fecal samples as well as some estimate of indigestibility of DM within the diet being fed. Of these 2 factors, starch content of the diet within the dairy data set proved slightly more variable than DM digestibility (CV of 21.9 vs. 14.4%) and thereby would have greater effect than diet digestibility on K_i . Such was not the case with feedlot diets (CV = 16.2 vs. 25.0%). If diet samples are submitted to an analytical laboratory simultaneously with fecal samples, starch or NDF content of the diet can be determined directly leading to more precise estimates of digestibility. Estimates of DM digestibility also can be derived from proximate composition of diet components based on TDN equations developed by Weiss et al. (1992) or the NRC (2001) or predicted from laboratory analyses. For feedlot cattle, NE values of diets also can be calculated from performance measurements (DMI, BW, and ADG) of cattle as outlined by NRC (1996) or Zinn and Shen (1998) equations. Total digestible nutrients or DM digestibility then can be calculated from NE_m or NE_g via ME values. Ideally, if feed as well as fecal samples are provided by a client, direct analysis for nutrient content in the TMR and feces, when combined with some direct measurement of digestibility, e.g., lignin or acid insoluble ash, can yield apparent total-tract digestibility estimates not only for energy components (starch, NDF, and nonstarch cell contents) but also for protein and the full spectrum of dietary minerals and vitamins.

Figure 16. Graphic depiction of the effect of diet concentration of a component and of diet digestibility on the K_i value.

IMPLICATIONS

Although total-tract starch digestibility and concentration of starch in feces are mathematically linked, 2 additional factors, starch content of the diet and digestibility of diet DM, are implicit components of this relationship. When these 2 factors are ignored, the relationship of starch digestibility to fecal starch concentration is imprecise. Because DM digestibility generally increases when starch digestibility increases, the relationship of starch digestibility to fecal starch mathematically must be curvilinear, not linear. When combined with DMI, direct measurement of total-tract digestibility based on diet and fecal analysis for diet components with highly productive ruminants can be used to evaluate feed processing efficiencies and to improve the precision of quantifying the amounts of energy and nutrients available for maintenance and production for cattle at dairies or feedlots.

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