Fecal starch as an indicator of total-tract starch digestibility by lactating dairy cows

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ABSTRACT

To test whether total-tract starch digestibility by lactating dairy cattle could be predicted accurately from concentration of starch in fecal dry matter (FS), data from 8 digestibility trials, 30 diets, and 564 individual starch measurements were compiled from trials conducted at the University of Wisconsin-Madison. Total-tract starch digestibility by individual cows was determined from the concentrations of starch in total mixed rations and feces (rectal grab samples) and concentrations of several external or intrinsic feed markers. Fecal starch concentration was closely and linearly related to total-tract starch digestibility ($R^2 = 0.94$). Differences in fecal sampling time for FS, such as day within week or week, did not influence determination of FS concentration. In contrast, time of day when fecal samples were taken influenced FS concentration, but the differences were minimal (mean $\pm$ standard error of the mean = 0.4 ± 0.1). These data suggest that on-farm collection of feces from individual cows or pens of cows may be sampled only once per day. Fecal pH was not related to FS when FS was only 1 to 3%, indicating that fecal pH was not a good index of FS and not practical as a tool to assess total-tract starch digestibility for lactating dairy cows when fecal starch excretion is low. An equation was developed to predict FS using near-infrared reflectance spectroscopy of dried ground fecal samples; the equation had moderate to good accuracy ($R^2 = 0.83$ – 0.94) and a low standard error of prediction. Fecal starch concentration can be used to monitor total-tract starch digestibility.

Key words: digestibility, lactating cow, starch

INTRODUCTION

Interest in improving starch digestibility by lactating dairy cows has been stimulated by the high cost of cereal grains. An increase in total-tract starch digest-

ibility (TTSD) can increase yield of milk and protein and feed efficiency (Firkins et al., 2001). Total-tract digestibility of starch in dairy cows ranges from 70 to 100% (Firkins et al., 2001; Ferraretto et al., 2013) and is influenced by numerous factors such as particle size, grain processing, and storage method (Firkins et al., 2001; Ferraretto et al., 2013); harvest maturity, moisture content, and duration of silo fermentation (Hoffman et al., 2011; Ferraretto and Shaver, 2012b); and corn endosperm type (Taylor and Allen, 2005; Lopes et al., 2009). Currently, one common method to estimate the amount of starch that escapes digestion is to sieve fecal material and qualitatively estimate the amount of grain caught by the screen (Hall, 2002). Combined with measurements of the dietary starch concentration and in vitro starch digestibility of feedstuffs, such measurements should lead to ration adjustments when the amount of grain found in feces is excessive (Hall, 2002). Commercial laboratories offer a wet chemistry measurement of starch concentration in feces; estimates of starch availability based on enzyme, yeast, or in vitro incubation; and quantitative measurements of total-tract nutrient digestibility, including starch, that require nutrient analysis of TMR and feces.

Fecal starch concentration (FS) proved to be an accurate indicator of TTSD by feedlot cattle (Zinn et al., 2002; Corona et al., 2005), and FS has been used to assess the effectiveness of grain processing (Zinn et al., 2007). Fernandez et al. (1982) first reported a relationship between TTSD and FS for lactating dairy cows. In a literature review, Owens and Zinn (2005) also reported a modest relationship ($R^2 = 0.73$) between FS and TTSD by lactating dairy cows.

More recently, J. D. Ferguson (University of Pennsylvania, Philadelphia, PA; personal communication), as referenced by Grant (2010), reported that TTSD could be estimated from FS in lactating dairy cows on commercial farms from samples of TMR and feces. Starch and lignin concentrations were determined for the TMR and pen fecal composites to calculate TTSD; a negative relationship between FS and TTSD was detected ($R^2 = 0.73$).

Experimental objectives of this research were to (1) develop an equation to estimate TTSD from FS mea-

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sured in controlled research trials; (2) determine the distribution and variation for FS concentrations associated with week, day within week, and time within day of sample collection; (3) examine the relationship between fecal pH and FS; and (4) evaluate the potential for developing a near-infrared reflectance spectroscopy (**NIRS**) technique and equation for quantifying FS.

MATERIALS AND METHODS

All experimental procedures and animal use protocols were approved by the Research Animal Resource Committee of the College of Agricultural and Life Sciences of the University of Wisconsin-Madison.

Fecal Starch Equation

To develop an equation to estimate TTSD from FS, data from 8 trials at the University of Wisconsin-Madison from 1997 to 2012 were compiled. These trials are described in Table 1. Across all trials, lactating Holstein cows ranged from 29 to 151 DIM at the start of the trials. Cow within each experiment served as the experimental unit except in the trial by Ferraretto et al. (2012), where gate feeders (RIC system, Insentec, Marknesse, the Netherlands; 1.40 m deep, 0.80 m wide, and 0.75 m high; 2 cows per gate) served as the experimental unit. All diets were fed as a TMR with daily DMI for individual cows or gates calculated as feed delivered daily minus daily refusals. Starch intake was corrected for the concentration of starch remaining in the refusals. Fecal grab samples were collected from each cow twice at 1000 and 2200 h during the last 3 d of each period for Bal et al. (1997) and at 1100 and 2300 h on d 24 through 26 for Schwab et al. (2002); or thrice at 1000, 1600, and 2200 h during the last 3 d of each period for Bal et al. (2000a) and at 0800, 1400, and 2000 h during the last 3 d of each period for Bal et al. (2000b). Six fecal grab samples were collected at 8-h intervals covering each 4-h clock period over 3 consecutive days during each period for Lopes et al. (2009), or during wk 8 of the treatment period for Gencoglu et al. (2010), or during wk 4 and 8 of the treatment period for Ferraretto et al. (2012). For all trials, TMR and fecal samples were dried at 60°C for 48 to 72 h in a forced-air oven and ground through a 1-mm screen with a Wiley mill (Arthur H. Thomas, Philadelphia, PA; Bal et al., 1997). To calculate digestibility from concentrations of nutrients in the TMR and feces, external or intrinsic marker techniques were employed (Table 1). Analysis of starch, rare-earth markers, lignin, and indigestible NDF were conducted as described in the individual papers. Apparent TTSD for each cow fed each diet in

each period was calculated using the following equation (Bal et al., 1997):

$$\text{Apparent TTSD (\% of DM)} = 1 - [(\text{TMR marker concentration/fecal marker concentration}) \times (\text{FS concentration/TMR starch concentration})].$$

The distribution of FS was determined using data from 15 trials conducted at the University of Wisconsin-Madison from 1997 to 2012. Trials included in the distribution plot were the 8 trials used to determine the TTSD equation, plus Ferraretto and Shaver (2012a), Akins et al. (2014), and 6 unpublished trials (Arndt et al., 2009; Ferraretto and Shaver, 2013; Fredin et al., 2013a,b; Lopes et al., 2013a,b). Distribution data included cow, gate, or pen as the experimental unit with samples obtained from continuous-lactation or Latin square trials. Fecal grab sampling methods and TTSD calculations for all trials were similar to procedures described previously. Starch concentration in feces was analyzed as described by Ferraretto et al. (2012) for the experiments by Ferraretto and Shaver (2012a), Akins et al. (2014), and the 6 unpublished trials (Arndt et al., 2009; Ferraretto and Shaver, 2013; Fredin et al., 2013a,b; Lopes et al., 2013a,b).

Fecal Starch Sampling Variation

The effect of fecal sampling time on FS for day within week, week, and time within day was determined within a trial conducted in our laboratory (Akins et al., 2014). One-hundred twenty-eight cows stratified by DIM (90 ± 33 DIM), breed (Holstein and Holstein $\times$ Jersey crossbred), and parity (primi- and multiparous) were assigned randomly to 1 of 16 pens with 8 cows per pen in the University of Wisconsin sand-bedded freestall barn (Emmons Blaine Dairy Research Center, Arlington, WI). Each pen contained 3 primiparous Holstein, 3 multiparous Holstein, and 2 multiparous Holstein $\times$ Jersey crossbred cows that were used in a continuous-lactation trial with a 4-wk covariate period followed by a 12-wk treatment period. All cows received the same diet during the covariate period followed by a change to 1 of 4 treatment diets during the test period. Diets were fed once daily as a TMR. Pens were supplied with TMR to allow 5% refusals, with daily DMI determined on a pen basis throughout the trial. Daily pen refusals were recorded and removed each morning before delivery of fresh feed. Fecal samples were collected just before feeding (0 h) and 12 h postfeeding during the last 2 d of the covariate period and in wk 6 and 12 of the treatment period. Fecal samples were

Table 1. Summary of trials used for the development of an equation to estimate apparent total-tract starch digestibility in lactating dairy cows from fecal starch concentration

Reference	n ¹	Diets	Average DIM at trial initiation	Treatment duration ² (d)	Dietary forage (% of DM)	Corn silage (% of dietary forage)	Dietary starch range (% of DM)	Digestibility marker ³
Bal et al., 1997	20	4	75	28	50	67	29–35	Yb
Bal et al., 2000a; trial 1	24	4	75	28	50	67	28–29	Yb
Bal et al., 2000a; trial 3	24	4	75	28	53–61	67	23–28	La
Bal et al., 2000b	24	4	71	28	50	67	31–32	La
Schwab et al., 2002	24	4	102	28	60	66	24–26	La
Lopes et al., 2009	6	3	67	14	55	0	19–21	iNDF
Gencoglu et al., 2010	36	3	51	105	50	66	21–27	iNDF
Ferraretto et al., 2012	32	4	114	98	50	75	21–31	Lignin

¹Number of experimental units.
²Experiments included continuous lactation or Latin square trials.
³Yb = ytterbium; La = lanthanum; iNDF = indigestible NDF.

collected from each cow in each pen by rectal grab and composited on a wet volume basis by pen.

Fecal pH

The relationship between FS and fecal pH was determined from 2 previous trials from our laboratory conducted in the University of Wisconsin sand-bedded freestall barn (Emmons Blaine Dairy Research Center, Arlington, WI). In the first trial (Ferraretto and Shaver, 2012a), 112 cows were stratified by DIM (116 ± 36 DIM), breed (Holstein and Holstein $\times$ Jersey crossbred), and parity (primi- and multiparous), and assigned randomly to 1 of 14 pens with 8 cows per pen. Pens were assigned randomly to 1 of 2 treatments in a continuous lactation feeding trial with a 2-wk covariate period, when cows were fed a single diet, followed by an 8-wk treatment period, when cows were fed 1 of 2 assigned treatment diets. Likewise, in the second trial (Ferraretto and Shaver, 2013), 112 cows stratified by DIM (139 ± 47 DIM) and parity (primi- and multiparous) were assigned randomly to 1 of 14 pens with 8 cows per pen. Pens were assigned randomly to 1 of 2 treatments in a continuous-lactation feeding trial with a 2-wk covariate period, with cows fed a single diet followed by a 10-wk treatment period with cows fed 1 of 2 treatment diets. In both trials, pens were supplied with TMR to allow for 5% refusals, with daily DMI determined on a pen basis throughout the trial. Daily pen refusals were recorded each morning before delivery of fresh feed. Fecal samples were collected 4 to 6 h postfeeding during the last day of wk 5 and 8 or wk 5 and 9 of the treatment period for the first and second trials, respectively. Fecal samples were collected from each cow in each pen by rectal grab and composited on a wet volume basis by pen. For fecal pH measurements, as described by Gressley and Armentano (2005), approximately 50 g of fresh feces and 25 g of distilled water were placed in a specimen cup. The cup was shaken for 20 s in a vortex, liquids were squeezed through cheesecloth, and the pH of the liquids was determined with a Cardy Twin pH meter (model B-213, Spectrum Technologies Inc., Plainfield, IL).

NIRS Equation

To test the potential for optical methods to quantify FS, NIRS scans were obtained from 295 fecal samples, 238 samples from 3 trials previously conducted at the University of Wisconsin-Madison (Ferraretto and Shaver, 2012a, 2013; Akins et al., 2014), and 57 additional samples obtained from Rock River Laboratory (Watertown, WI). Fecal samples from Rock River Laboratory were added to increase the number of samples that

had >5% FS. All fecal samples from the University of Wisconsin-Madison were dried and ground to pass a 1-mm Wiley mill screen, with fecal starch content determined as described previously. The samples from Rock River Laboratory were ground to pass through a 1-mm screen with a Udy mill (Udy Corp., Boulder, CO). Each fecal sample was packed into a cylindrical sample holder equipped with a quartz window and scanned between 400 and 2,498 nm, as described by Mentink et al. (2006), using an NIRS (model 6500; Foss-NIR System, Silver Spring, MD). Spectra were retained for statistical analysis.

Statistics

The REG procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC) was used to analyze the relationship between FS and TTSD or fecal pH. To determine if a mean or linear bias was present in the regression model, the residuals (observed – predicted) were evaluated against predicted values centered at the mean (St-Pierre, 2003). Due to the presence of heteroscedasticity in the regression model for FS and TTSD, a default heteroscedasticity-consistent matrix estimator was included by adding the WHITE option to the MODEL statement to include heteroscedasticity-consistent standard errors (White, 1980). Fecal starch variation data (Akins et al., 2014) were analyzed as a completely randomized design using Proc MIXED with the data from the preliminary period being used as a covariate and week as a repeated measure using the first-order autoregressive covariance structure, which provided the best fit according to Sawa's Bayesian information criterion. The model included day, sampling time, week, and their interactions as fixed effects, and pen as a random effect. Degrees of freedom were calculated using the Kenward-Roger option. Data are reported as least squares means. All statistical significance and trends were declared to be significant at $P \leq 0.05$ and a trend when $P > 0.05$ to $P \leq 0.10$, respectively.

Near-infrared reflectance spectroscopy calibrations were developed using WinISI software (version 3.0; Infrasoft International LLC, State College, PA). Calibrations for FS using NIRS were developed using various math transformations and a different number of coefficients in the models as defined by Shenk and Westerhaus (1991a). Calibration performance was evaluated using cross validation procedures (Shenk and Westerhaus, 1991b) in which the calibration coefficient of determination and prediction error was challenged by dividing the calibration samples into 4 subsets of equal size, one subset being reserved for validation and the remaining subsets being used for calibration. Strength of the calibration was based on the cross-validation

coefficient of determination (R^2), the standard error of performance (SEP) corrected for bias, and the mean bias.

RESULTS AND DISCUSSION

Fecal Starch Equation and Variation

The trials used to predict TTSD from FS are described in Table 1. Forages used in the trials were alfalfa silage ($n = 1$) or corn silage and alfalfa silage ($n = 7$). The mean dietary starch concentration across all experiments was 27% of DM (range = 19–35%). Mean CP and NDF concentrations (data not presented) were 17% (range = 15–20%) and 28% of DM (range = 23–37%), respectively. This wide range in dietary nutrient composition indicates that the data set should provide a robust equation to estimate TTSD from FS concentration.

The relationship between FS concentration and TTSD is presented in Figure 1A. We observed a strong linear relationship between TTSD and FS: $\text{TTSD}\% = 100.0\% - (1.25 \times \text{FS}\%)$; $R^2 = 0.94$; $P < 0.001$. Fernandez et al. (1982) predicted TTSD from FS for lactating cows fed high-starch diets (mean = 34% of DM) in which sorghum grain and silage were the only starch sources; $\text{FS}\% = 59.9\% - (0.55 \times \text{TTSD}\%)$; $R^2 = 0.64$. Rearranging terms, this equation becomes $\text{TTSD}\% = 108.8\% - (1.82 \times \text{FS}\%)$. The greater mean starch concentration of the diets in the trial by Fernandez et al. (1982) likely resulted in a TTSD estimate from FS that was lower than observed in the present study. The residuals (observed – predicted) were plotted against centered predicted TTSD values to determine if a mean or linear bias was present in the regression model (Figure 1B). The mean predicted value used to center the data was 93.5. The mean bias and slope bias were nonsignificant, indicating that FS can be used to accurately predict TTSD.

The relationship observed in the present study is comparable with estimates of TTSD in dairy cows by Owens and Zinn (2005), where $\text{TTSD}\% = 98.2\% - (0.93 \times \text{FS}\%)$; $R^2 = 0.73$. The relationship is also comparable with estimates of TTSD by steers fed whole, ground, and steam-flaked corn ($R^2 = 0.97$, Corona et al., 2005; $R^2 = 0.96$, Zinn et al., 2007). However, the slope of the equation in the present study was not as steep as in the equation presented in Grant (2010) for dairy cows, where $\text{TTSD}\% = 98.7 - (1.76 \times \text{FS}\%)$; $R^2 = 0.78$. The relationship described in Grant (2010) was developed from a data set containing 72 samples from lactating and dry cows collected from 8 farms. A lower measured TTSD may have resulted in steepness of the slope for the relationship between FS and TTSD. This

Figure 1. Regression plot of fecal starch (% of DM) and total-tract starch digestibility (% of starch intake) for individual cows. (A) Total-tract starch digestibility % = $100.0 (\pm 0.07) - 1.25 (\pm 0.03) \times \text{fecal starch \%}$; root mean square error (RMSE) = 0.936, $R^2 = 0.94$, $P < 0.001$, $n = 564$; (B) plot of the residuals: $Y = -0.05 (\pm 0.04) + 0.00 (\pm 0.01) \times (X - 93.5)$; RMSE = 0.94, $R^2 = 0.00$, $P = 0.93$, $n = 564$.

may have occurred through dilution of FS due to a greater dietary NDF content of the diet, a lower NDF digestibility, or both.

Fecal starch concentration accounted for 94% of the variation in TTSD in the present study. It is unlikely that total-tract digestibilities of other nutrients are related as directly to their concentrations in feces. For example, based on a subset of the TTSD data in the present study, where measurements of total-tract NDF digestibility were available, the calculated correlation coefficient for the relationship between total-tract NDF digestibility and fecal NDF concentration was low: total-tract NDF digestibility = $100.0 - (1.26 \times \text{fecal NDF\%})$; $R^2 = 0.18$; $n = 390$ (data not provided in figure). Total-tract digestibility of NDF was not well predicted from fecal NDF concentration. Total-tract digestibility of CP and fat would be under-predicted from fecal concentrations of CP and fat because of the contribution of endogenous and microbial synthesis of these 2 nutrients. Although NDF is not synthesized endogenously or by microbes, NDF has a lower total-tract digestibility than starch (NRC, 2001). In the present study, fecal starch content was typically 0 to 5%, and

TTSD was typically greater than 95%, resulting in most observations of TTSD occurring at or near the y-intercept (100% TTSD digestibility). This resulted in a low coefficient of variation for FS at or near the intercept, where most of the observations occur. Because NDF is not as digestible as starch, the coefficient of variation of fecal NDF concentration was rather high and the relationship of total-tract NDF digestibility to fecal NDF concentration was quite poor.

The distribution of FS observed in collective studies is presented in Figure 2. Fecal starch averaged 3% across all trials, with 60% of the fecal samples ranging from 0 to 3% starch. In the data set used to develop the aforementioned TTSD equation, 48% of the samples ranged from 0 to 3% FS. Although the data from the 8 trials used to create the TTSD equation are represented in the distribution data set, observations ranging from 0 to 3% FS are underrepresented in the TTSD equation. To determine if the underrepresentation of samples ranging from 0 to 3% FS statistically enhanced the TTSD equation, 119 samples with >3% FS were chosen, at random, and excluded from the data set. This resulted in 61% of the new data set ranging from 0 to 3% FS, similar to the percentage of observations in the distribution plot. The resulting regression equation was unaffected: $\text{TTSD\%} = 99.9 - (1.24 \times \text{FS\%})$; $R^2 = 0.94$, suggesting that the original equation was not altered by the smaller number of samples that contained 0 to 3% FS.

Fecal Starch Sampling Variation

The effect of sampling time on FS concentration for day within week, week, and time within day are presented in Figure 3. Fecal starch concentration was unaffected by sampling day within week ($P = 0.14$) or by week ($P = 0.11$). However, FS was greater in samples collected 0 h postfeeding compared with 12 h postfeeding (2.0 vs. 1.6%; SE = 0.11; $P < 0.01$), although the numerical difference was small (0.4 percentage units). We observed an effect for the interaction between fecal collection time and day of sampling on FS (Figure 4; $P < 0.01$). Fecal starch concentration decreased 12 h postfeeding compared with samples collected just before feeding (0 h) on 3 of 4 d, but increased on one of the days. Leonard et al. (1989) measured FS over 3 time courses (0–8, 8–16, and 16–24 h) after 1 d of total fecal collection from Angus steers (6 steers per treatment) fed hay with ground corn once per day. Fecal starch concentration (mean $\pm$ SE) was 7.8 ± 1.9 , 4.8 ± 1.1 , and $10.3 \pm 2.0\%$ for 0–8, 8–16, and 16–24 h, respectively. Average FS over 24 h was $7.6 \pm 1.2\%$. Statistical analysis on the effect of sampling time within day for differences in FS was not performed in

Figure 2. Histogram of fecal starch as a percentage of DM, collected from 15 trials conducted at the University of Wisconsin-Madison; mean = 3.1; minimum = 0.1; maximum = 16.1; n = 1,036. The frequency of observations is listed above for each range.

that experiment. In the trial by Leonard et al. (1989), FS appeared to vary during a day. However, because a greater amount of corn grain was fed to their steers (31% of DMI), FS was increased. Observations from the present study, where TTSD was high (mean = 97%), indicate that differences in FS may occur over the course of one day, but that these differences were numerically small. Therefore, a single time point with multiple cows sampled should be suitable for fecal collections on farms to determine TTSD for routine monitoring or diagnostic purposes. Further research may be warranted to determine if a lower TTSD increases the variability of FS during a single day.

Fecal pH

We found no relationship (data not provided in figure) between FS and fecal pH within either trial 1 (FS = 1.5; $P > 0.10$; $R^2 < 0.01$; Ferraretto and Shaver, 2012a) or 2 (FS = 3.0; $P > 0.10$; $R^2 < 0.14$; Ferraretto and Shaver, 2013) of this study. The published literature is inconsistent in regards to this relationship. For example, Wheeler and Noller (1976) observed that lower fecal pH coincided with higher FS concentrations in growing heifers and lactating dairy cows. Likewise, a strong negative relationship between FS and fecal pH was observed for steers and sheep (Wheeler and Noller, 1977). Others have observed either no relationship (Erdman et al., 1982; Fernandez et al., 1982, experiment 3), a slight relationship (Erdman et al., 1980; Kilmer et al., 1980, 1981), or an intermediate relationship (Fernandez et al., 1982, experiment 1; Ire-

land-Perry and Stallings, 1993) between FS and fecal pH in dairy cows. Fecal pH can be affected by factors other than starch, including dietary buffers, buffering capacity of forages, dietary forage concentration, and ruminal acidosis (Erdman et al., 1982; Nocek et al., 1983; Gressley et al., 2011). Gressley et al. (2011), in a recent review of the literature, suggested that hindgut acidosis caused by starch is a function of the amount of starch (kg/d) flowing to the large intestine and its rate of fermentation. In Ferraretto and Shaver (2012a), approximately 69% of the starch was from processed corn silage, whereas in Ferraretto and Shaver (2013), approximately 76% of the starch was from processed corn silage and high-moisture corn. Processed corn silage and high-moisture corn characteristically have high ruminal starch digestion (Ferraretto and Shaver, 2012b; Ferraretto et al., 2013). Abomasal infusions of large doses of starch (~4 kg/d) induced decreased fecal pH (Gressley et al., 2011), and consequently, the amount of starch flowing to the large intestine in the present study may have been inadequate to increase cecal fermentation to cause a decrease in fecal pH. Under the conditions of present experiments, when FS was 1.5% in Ferraretto and Shaver (2012a), and 3.0% in Ferraretto and Shaver (2013), fecal pH was not reliable as an indicator of FS and thereby not practical as a tool to assess TTSD by dairy cows. These findings are in agreement with Fernandez et al. (1982), who also reported a weak relationship between fecal pH and TTSD in either dry or lactating dairy cows where FS excretion was much greater compared with that in the current study (mean = 10.2% FS).

Figure 3. Effect of fecal sampling day, week, and time on fecal starch concentration (% of DM). (A) Fecal sampling by week and day: SEM = 0.15, $P = 0.14$; (B) Fecal sampling by week: SEM = 0.11, $P = 0.11$; (C) fecal sampling by time of day postfeeding: SEM = 0.11, $P < 0.01$.

NIRS Equation

Near-infrared spectroscopy calibration and cross-validation statistics of FS predictions are presented in Table 2. Fecal starch concentration was predicted by NIRS with good accuracy for all math transformations except for 2, 2, 2, 1. Combined, simple 1, 4, 4,

Figure 4. Effect of fecal sample collection time on fecal starch concentration (% of DM). Fecal samples were collected just before feeding at 0 h or 12 h postfeeding on wk 6 (◆) and 12 (□) for 2 consecutive days. Time $\times$ day interaction = $P < 0.01$, SEM = 0.18. Time $\times$ week $\times$ day interaction = $P < 0.01$, SEM = 0.25.

1 math transformations yielded a robust coefficient of determination ($R^2 = 0.94$) and a low standard error of calibration (SEC = 0.45) for predicting FS. Cross validation statistics indicated that NIRS calibrations for FS were robust and remained largely unaltered when cross validated. The coefficients of determinations for calibration and cross validation were similar ($R^2 = 0.94$ vs. 0.91) for the 1, 4, 4, 1 math transformations. Likewise, SEC and SEP were similar at 0.45 and 0.57. The relationship between FS and NIRS-determined FS using the 1, 4, 4, 1 transformation was predicted with good accuracy for all samples: NIRS-predicted FS% = $0.4 + (0.07 \times \text{FS}\%)$; $R^2 = 0.88$; $P < 0.001$; Figure 5A. The coefficient of determination ($R^2 = 0.88$) presented in Figure 5A is lower than the cross-validation coefficient of determination ($R^2 = 0.92$) for the 1, 4, 4, 1 transformation reported in Table 2 because 23 outlying observations were omitted during the calibration process; the residuals are plotted in Figure 5B. The mean predicted value used to center the data was 2.99. The mean bias and slope bias were nonsignificant, indicating that NIRS can be used to accurately predict FS. The omitted observations were left in Figure 5A and B to demonstrate that NIRS accurately predicted FS when FS is from 0 to 5%. However, when assayed FS was greater than 5%, FS tended to be poorly predicted by NIRS. More samples greater than 5% FS need to be added to the NIRS sample set to improve this prediction when FS is high or when TTSD is lower than samples included in this sample set.

Starch is routinely predicted by NIRS in forages and TMR with high accuracy and precision (Barrière et al., 2004; Mentink et al., 2006). Most research on rumi-

Table 2. Calibration and cross-validation statistics for near-infrared reflectance spectroscopy analysis of fecal starch

Transformation ¹	Calibration			Cross-validation		
	n	Starch, % of DM	SEC ²	R ²	R ²	SEP ³
1, 4, 4, 1	312	2.69	0.45	0.94	0.91	0.57
1, 2, 2, 1	316	2.76	0.45	0.95	0.90	0.64
1, 10, 10, 1	317	2.84	0.50	0.95	0.94	0.58
2, 4, 4, 1	320	2.76	0.45	0.95	0.90	0.63
2, 2, 2, 1	319	2.72	0.54	0.92	0.83	0.78
2, 10, 10, 1	316	2.71	0.49	0.94	0.91	0.60

¹Spectral math transformation values equal order of derivative function, gap width, segment length of first smoothing, and segment length of second smoothing. Second smoothing of spectra was not conducted; therefore, all values are 1.

²SEC = standard error of calibration.

³SEP = standard error of prediction.

nant animals regarding fecal nutrient NIRS predictions has focused on predicting dietary nutrient composition from feces (Lyons and Stuth, 1992; Boval et al., 2004) or predicting OM digestibility from feces (Lyons and Stuth, 1992; Boval et al., 2004; Franchone et al.,

2009) with moderate coefficients of determination ($R^2 = 0.72\text{--}0.85$). In total, the accuracy and precision of NIRS prediction of FS appears robust and would allow for a rapid and inexpensive measurement of FS for farm-level determination of TTSD. In fact, several commercial laboratories have recently begun offering NIRS-predicted FS.

CONCLUSIONS

Fecal starch concentration proved accurate as a predictor of TTSD by individual dairy cows in a series of controlled experiments where animals were fed corn silage-based diets. In addition, sampling day, week, or time within day had minimal influence on FS when TTSD was high. Because of the relatively minimal numerical differences on FS for sampling time within day, composites of fecal samples collected from multiple cows in pens once per day may provide a representative sample of feces for measuring FS. Based on measurements when TTSD was high, fecal pH was not reliable as an indicator of FS. Fecal starch concentrations can be accurately predicted by NIRS, allowing estimates of TTSD to be determined rapidly and economically. Fecal starch appears useful to monitor the TTSD of a ration to detect and subsequently alter rations with suboptimal TTSD. Diet alteration, in turn, should help to improve TTSD and efficiency of milk production.

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Figure 5. Regression plot of fecal starch content (% of DM) determined by wet chemistry and near-infrared spectroscopy (NIRS). (A) NIRS-predicted fecal starch % = $0.4 (\pm 0.07) + 0.83 (\pm 0.02) \times$ fecal starch %; root mean square error (RMSE) = 0.83, $R^2 = 0.88$, $P < 0.001$, $n = 335$; (B) plot of residuals: $Y = 0.00 (\pm 0.05) + 0.00 (\pm 0.02) \times (X - 2.99)$; RMSE = 0.83, $R^2 = 0.00$, $P = 1.00$, $n = 335$.

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