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Development of NIRS calibrations to estimate fecal composition and nutrient digestibility in beef cattle

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Jancewicz, L. J., Swift, M. L., Penner, G. B., Beauchemin, K. A. Koenig, K. M., Chibisa, G. E., He. M. L., and McAllister, T.A. 2016. **Development of NIRS calibrations to estimate fecal composition and nutrient digestibility in beef cattle.** Can. J. Anim. Sci. **XX**: YY-ZZ. Feces from cattle fed 65 different diets and from 23 digestibility studies were used to develop near infrared spectroscopy (NIRS) calibrations to predict fecal composition [organic matter (OM), starch, nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), and crude fat (CF)] and digestibility [DM, OM, starch, crude protein (CP), NDF, ADF, and gross energy (GE)]. The prediction of these parameters in feces collected from cattle fed wheat or barley grain (89% of diet DM) with barley silage (study 1), or barley grain diets containing varying levels of barley silage (study 2) was assessed. Validations for feces composition displayed moderate to excellent linearity ($R^2_{\text{val}} > 0.70$) for OM, starch, N, NDF, and ADL, and poor linearity for ADF and CF ($R^2_{\text{val}} = 0.25$). When both digestibility and feedlot datasets were combined, cross-validation for fecal composition found accurate predictions ($R^2_{\text{cv}} > 0.90$, $\text{SECV} < 2.42$) for all fecal constituents except fat. Accuracy of predicting digestibility was high for starch ($R^2_{\text{cv}} = 0.84$, $\text{SECV} = 1.06$), moderate for DM, OM, CP, and GE ($R^2_{\text{cv}} > 0.71$, $\text{SECV} < 2.88$), but poor for NDF and ADF ($R^2_{\text{cv}} < 0.33$, $\text{SECV} > 7.86$). We conclude that fecal NIRS can predict both chemical composition and digestibility of most nutrients in feedlot cattle feces.

Key words: near infrared spectroscopy, feces, fecal composition, apparent total tract digestibility, feedlot cattle

Abbreviations: ADF, acid detergent fibre; ADL, acid detergent lignin; aTTD, apparent total tract digestibility; CF, crude fat; CP, crude protein; DM, dry matter; DMI, dry matter intake;

GD, global distance; **GE**, gross energy; **NIRS**, near infrared spectroscopy; **N**, nitrogen; **ND**, neighbourhood distance; **NDF**, neutral detergent fibre; **OM**, organic matter; **PCA**, principle component analysis; R^2_{cal} , coefficient of determination of calibration; R^2_{cv} , coefficient of determination of cross-validation; R^2_{val} , coefficient of validation; **SEC**, standard error of calibration; **SECV**, standard error of cross-validation; **SEP**, standard error of prediction.

For Review Only

Introduction

Traditional laboratory methods for determining the chemical composition of feed or feces for accurate estimation of diet digestibility are impractical in commercial feedlots where results are required immediately for feeding management decisions. Near infrared spectroscopy (NIRS) is a rapid alternative to wet chemistry for predicting the composition of feeds and feces and can be used to estimate the digestibility of diets fed to ruminants (Dixon and Coates 2009).

Traditional methods of estimating diet digestibility require data on feed intake, diet and fecal composition and excretion. The success of using NIRS of feces to predict digestibility relies on the principle that there is sufficient spectral information in feces to describe the composition of the diet ingested, despite the process of removal and addition of organic compounds through digestion and endogenous secretions (Dixon and Coates 2009). Near infrared spectroscopy has been employed to predict the chemical composition of manure (Chen et al. 2013), nutrient intake of cattle grazing pastures (Dixon and Coates 2009) and the organic matter (OM) digestibility of a variety of forage (Coleman et al. 1995; Boval et al. 2004), and concentrate diets (de la Roza et al. 2002; Garnsworthy and Unal 2004). Calibrations have also been developed for rapid measurement of starch concentration in feces and to predict starch digestibility by dairy cattle (Fredin et al. 2014). Application of NIRS in a similar manner in feedlot cattle production could also have considerable value as levels of fecal starch are highly correlated to starch digestibility (Zinn et al. 2002). When grain is not thoroughly digested, high fecal starch concentration or low starch digestibility could imply inadequate grain processing. In combination with fecal starch, fecal N has been shown to improve the predictability of starch digestibility (Zinn et al. 2007). The consequences of fecal nutrients for digestibility has not been intensively studied under production conditions, but associations have been found between fecal

nutrients and the growth of cattle fed inadequately processed wheat (Jancewicz et al. 2016, submitted).

The objective of this research was to generate NIRS calibration models for predicting fecal composition and apparent total tract digestibility (aTTD) of nutrients by feedlot cattle. Digestibility of a range of feedlot diets was estimated using total collection procedures and the data was used to develop NIRS calibrations. The robustness of these calibrations was subsequently assessed by collecting feces from feedlot cattle fed in research and commercial production settings.

Materials and Methods

Origin of samples

Fecal samples for the development of NIRS calibrations to predict fecal composition (10 studies; Table 1) and apparent total tract digestibility (aTTD; 8 studies; Table 2) were obtained from digestibility studies where cattle were fed diets differing in grain type, grain processing, forage type and proportion, and level and type of by-product as cited in Tables 1 and 2. Studies were conducted at the University of Saskatchewan, the Lethbridge Research and Development Centre, and commercial feedlots around Lethbridge, Alberta over a 7 year period. Calibrations to estimate fecal composition (Table 1) and aTTD (Table 2) were validated using samples collected from growth performance feedlot studies.

NIRS calibrations for chemical composition

Collection of samples

For digestibility experiments, cattle were housed in individual stalls and samples were collected (≥ 250 g wet weight) either from the rectum or off a clean floor shortly after defecation. Samples were collected from individual animals, and pooled across days (4 to 5 d)

within each collection period during the metabolism studies. For feedlot experiments, cattle were housed in group pens outdoors, and subsamples from 4 fresh fecal pats were composited equally by wet weight (400 g). For fecal composition, feedlot samples were collected over 1 d, and for aTTD validations, samples were collected at 3 or 4 wk intervals over the feeding period. In addition, 10 fecal samples were collected in a similar manner from one feedlot, and spiked with increasing levels of whole barley grain to expand the range of samples that represented the levels of starch ($\geq 19\%$ of fecal DM) that occur in feces from cattle fed inadequately processed grain (Jancewicz et al. unpublished).

All fecal samples were dried at 55°C and ground through a 1 mm screen using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) or through a 0.75 mm screen using a Retsch grinder (Verder Scientific, Inc, Newton, PA) depending on the location that the original study was conducted. Ground samples were then packed into quartz ring cups (25 g) and scanned in duplicate (two repacks; where the second scan was a completely different sup-sample from the first) using a SpectraStar Near-Infrared analyzer 2400 RTW (Unity Scientific, Brookfield, CT). Spectral information was collected at wavelengths between 1200 and 2400 nm in 1 nm increments. Duplicate spectra of each sample were averaged. To account for small differences in particle size as a result of using different grinders, standard scatter corrections were applied to the spectra as described below.

Reference analysis

Calibrations were developed using samples collected from digestibility studies and then were used to assess fecal samples from feedlots. Fecal samples were analyzed for analytical DM [Association of Official Analytical Chemists (AOAC) 2005, method 930.15] and OM (AOAC 2005, method 942.05). Samples were further ground using a ball mill (Mixer Mill MM2000,

Retsch, Haan, Germany) for determination of starch and nitrogen (N). Starch concentration in the compiled studies was determined using two methods either by enzymatic hydrolysis of α -linked glucose polymers as described by Rode et al. (1999) with modifications according to Vyas et al. (2014), or a glucoamylase enzymatic reaction followed by oxidation in a YSI 2700 Biochemistry Analyzer (Yellow Springs, OH) with hydrogen peroxide detected on a platinum electrode surface (www.ysilifescience.com). Nitrogen was estimated by flash combustion, followed by gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy) or a LECO N analyzer (Joseph, MI) as described by Watson et al. (2003). Neutral detergent fibre and ADF were determined sequentially using an ANKOM fiber analyzer (ANKOM technology Corp. Fairport, NY) or the traditional Van Soest et al. (1991) procedure using filter glass crucibles. Heat stable α -amylase and sodium sulphite were included during the NDF analysis. Acid detergent lignin (ADL) was extracted using 72% sulphuric acid after the ADF procedure, followed by ashing at 550° C (Van Soest et al. 1991). Crude fat (CF; AOAC 2005, method 2003.05) was extracted by Soxtec HT6 System (Foss, Eden Prairie, MN) using anhydrous diethyl ether, followed by drying.

Spectral database

The full database contained spectra for 1110 fecal samples, representing 65 different diets and was divided into 601 spectra associated with the digestibility dataset, and 509 spectra in the feedlot dataset. The digestibility dataset contained the spectra with the corresponding reference analysis for constituents of interest including, OM, starch, N, NDF, ADF, ADL, or CF. However, not all studies had all analytical constituents of interest and as a result the number of samples contributing to the calibration varied among measured nutrients.

Fecal NIRS calibration development

Partial least squares regression (PLS) in UCal (Unity Scientific, 2010) was used to develop the calibrations. Two mathematical treatments were tested in the development of the calibrations: 1, 8, 8, 1 and 2, 8, 8, 1; where the first digit is the order of the derivative, the second is the gap over which the derivative was calculated, the third is the number of data points used in the running average for smoothing of derivative spectra, and the fourth is the number of data points over which the second smoothing was applied (ISI, 1999). Derivative spectra were used to emphasize small or large absorption peaks, and minimize overlapping peaks and baseline correction (Giese and French 1955). The standard normal variate (SNV) scatter correction was applied, along with the detrend (DT) function (Barnes et al. 1989).

The quality of calibrations was expressed by the coefficient of determination (R^2_{cal}), defined as the proportion of variability in the reference values accounted for by the regression equation (linearity and precision), and by the standard error of calibration (SEC) defined as the variability in the difference between reference and predicted values (Landau et al. 2015). As per Landau et al. (2015), the most important estimates of calibration quality were generated using cross validation in which a subset, typically one fifth of the total samples, were randomly selected and used to validate calibrations generated using the remaining four fifths of samples. The optimum math treatment for each constituent was identified on the basis of the greatest coefficient of determination of cross validation (R^2_{CV}), and the minimum standard error of cross validation ($SECV$, calculated as the average SEC of every subset). An evaluation based on the $SECV$ as an indicator of predictive accuracy may differ for each calibration as it depends on the prediction error, the purpose for which the predictions were to be used and the probability of a prediction being within the designated error (Coates and Dixon 2011). Based on a normal Gaussian distribution, a $SECV$ of 2.0 fecal OM % units (% of fecal DM) would indicate a

prediction error of ≤ 2.0 OM % units for 67% of samples, and ≤ 4.0 OM % units for 95% of samples. For other constituents, where the units are much smaller such as N, an *SECV* of 0.2 fecal N % units was considered acceptable. For fecal constituents, a $R^2_{CV} > 0.90$ was considered excellent, $0.80 < R^2_{CV} < 0.90$, good, $0.70 < R^2_{CV} < 0.80$, moderate; and $R^2_{CV} < 0.70$ poor.

Validation was performed by removing one study at a time to gage how each study affected the calibrations, and also to attempt to remove any large laboratory bias. The most robust calibrations were used to predict OM, starch, N, NDF, ADF, ADL, and CF in fecal samples collected from feedlots.

Sample selection of feedlot dataset for evaluation of calibration

To remove repetitious spectra and reduce the large number of samples requiring reference analysis in the feedlot database, samples in the validation set were predicted using the initial NIRS calibration, and ranked from the highest to lowest for each constituent (% of fecal DM). From a total of 509 samples, between 45 and 60 samples per constituent were selected to encompass the entire concentration range for all measured constituents, ensuring that at least one sample from every diet was represented in the dataset. Reference analysis was conducted on these samples as described above.

Evaluation of initial calibration and expansion of calibration

Regression standard errors of performance (*SEP*) were used as measures of predictive accuracy in the validation set. As per Coates and Dixon (2011), the coefficient of validation (R^2_{val}) was also used as a measure of linearity, with consideration for the large effect that differences in range of fecal constituents can have on R^2_{val} estimates. The slope and bias were reported, indicating the deviation from a 1:1 relationship, as well as the difference between actual and predicted NIRS values.

The degree that a predicted value matched its reference value was assessed by estimating the T limit (Coates and Dixon. 2011):

$$[T[x] = (\text{LabResult}[x] - \text{PredResult}[x] - \text{meanDifference})/(\text{Stdev of Differences})],$$

where $T[x]$ is the T limit for sample x ; LabResult is the reference value determined from wet chemistry; PredResult is the predicted value as determined by near infrared spectroscopy; meanDifference is the average difference between all reference and predicted values in the dataset; and Stdev of Differences is the standard deviation of all differences in the dataset.

Samples with $T > 2.5$ were designated as outliers that could have arisen as a result of poor NIRS prediction, error in the reference method, or bias in wet chemistry measurements.

Spectra were imported into Unscrambler ® X version 10.3 (CAMO software, Oslo, Norway), and a scatter plot of principle component (PC) scores for each sample within the metabolism and feedlot datasets were plotted along the first two principal components (x axis = PC1, y axis=PC2). Principle component analysis (PCA) is used to visualize the differences in spectral populations similar to Malley et al. (2005), where PCA was used to demonstrate differences in raw, stockpiled or composted manure. A mathematical treatment was applied to the spectra for PCA analysis (1, 8, 8, 1), and the standard normal variate (SNV) scatter correction and detrend (DT) functions. To identify outliers, principle component analysis established if a sample was contained within the calibration spectral population using the Mahalanobis statistic (Maesschalck et al. 2000) or H distance (> 3.0). In UCal, the global distance (GD) and the neighbourhood distance (ND) were used to identify outliers based on criteria similar to the Mahalanobis stastitic. The GD measured the distance between the spectrum of individual samples and the mean of the full database, with a $GD > 5.0$ being indicative of a sample that was outside the range of the predictive model. The ND calculated the position of the spectrum of

each sample within the database, and assessed the position of the sample of interest relative to all other samples. UCal used the closest ND measurement to differentiate unknown samples from known samples in the calibration set. If the ND was above a threshold of > 0.60 , the unknown sample was selected as a candidate to further expand the calibration dataset.

Apparent total tract digestibility

Collection of samples

Fecal samples used to generate digestibility calibrations were collected from individually penned cattle over a 4 to 5 d period after they were adapted to the diets for at least 10 d.

Subsamples of feces (400 g) were collected from daily total fecal output from each animal. Dry matter intake of each animal was measured and samples of diets and orts were collected for analysis. The majority of the digestibility studies were replicated Latin square designs using 8 cattle. One study was a completely randomized design with 6 cattle fed two diets over the backgrounding and finishing periods (Jancewicz et al. 2016). Fecal samples from digestibility experiments were dried, ground, and scanned as described above. More detailed information regarding these studies can be found in the references cited in Table 2.

Reference analysis

Fecal and feed samples from each experiment were analyzed using the same procedures as for the feces and used to estimate aTTD of nutrients and energy. Gross energy (GE) was measured by combustion using a bomb calorimeter (model E2k; Cal2k, Johannesburg, South Africa). For aTTD determination, subsamples of ingredients and any remaining orts were composited over total collection periods, dried (55 °C for 72 h) and analyzed, and in combination with DMI and total fecal output used to estimate aTTD of DM. Apparent total tract digestibility of OM, starch,

NDF, ADF, and GE was calculated from total nutrient (or GE) ingested (taking into accountorts) minus the nutrient (or GE) output in feces using the equations of Merchen (1988).

Spectral database

The database contained the spectra of 172 fecal samples, representing 23 diets for the development of NIRS calibrations for predicting aTTD of nutrients and energy of the diets consumed. The spectrum of each sample was associated with the corresponding aTTD coefficient for each nutrient and GE prior to calibration development.

Fecal NIRS calibration development and validations

The development of fecal NIRS calibrations for use in the estimation of aTTD coefficients was similar to that described above for chemical composition. A process of external validation was used to determine the influence of various factors on digestibility predictions by removing one study at a time, or classifying studies based on common attributes such as level of grain in the diet (>68% vs <44%) or grain type (barley vs corn). Prediction statistics were computed for these sub-attributes. The most robust NIRS calibrations based on highest R^2_{CV} and $SECV$ were selected to predict aTTD of DM, OM, starch, CP, NDF, ADF and GE in the samples selected from the feedlot database for validation (Table 2). For digestibility, previous designations for acceptable calibrations were more lenient, and $R^2_{CV} > 0.70$ was considered good.

Apparent total tract digestibility predictions of feedlot samples from validation studies 1 and 3 were analyzed using the PROC MIXED function for repeated measures since samples were collected at 3 or 4 wk intervals. Study 1 was analyzed as a factorial design, where the main factors included the effect of grain type, processing index, day and associated interactions, and pen as experimental unit. Study 2 was analyzed as a mixed linear model with silage level as a fixed effect, and pen as the experimental unit.

Data for aTTD of nutrients in the metabolism studies were analyzed using the mixed model procedure of SAS 9.1.(SAS,2004) for replicated 2×2 (Study 1) or 4×4 (Study 2) Latin square design with grain type as the fixed effect and square, animal within square and period within square as random effects for Study 1 with silage level considered a fixed effect for Study 2.

For comparison between aTTD determined in feedlot and digestibility studies, and determined using equations of Zinn (2002, 2007), the MIXED procedure was used with method (NIRS versus Actual or NIRS versus Zinn), diet, and the interaction of method $\times$ diet as fixed effects. Differences with $P < 0.01$ were declared significant.

Results

Calibration for chemical composition of feces

The validation steps conducted for the NIRS calibrations using the digestibility dataset resulted in the removal of chemical constituents that were poorly predicted in some of the selected studies, with these being removed and reported as missing values in the dataset. Where it was determined that values were deemed as outliers due to laboratory bias, technicians, or techniques, they were not included in the final NIRS calibrations. Once removed, additional validations conducted by removing one study at a time indicated that there was no benefit in developing calibrations that were specific for certain diets, grain types or grain processing methods using the current dataset (data not shown).

The 1, 8, 8, 1 spectral transformation was used for all calibrations as it produced calibrations with the highest R^2 and lowest SEC values. The calibrations yielded good to excellent linearity between reference and predicted values for OM ($R^2_{CV}=0.83$), starch

($R^2_{CV}=0.87$), N ($R^2_{CV}=0.97$), NDF ($R^2_{CV}=0.90$), ADF ($R^2_{CV}=0.93$), ADL ($R^2_{CV}=0.85$), but were poor for CF ($R^2_{CV}=0.43$; Table 3). Accuracy was excellent to acceptable for all constituents with *SECV* values of 0.96% for OM, 1.38% for starch, 0.11% for N, 2.41% for NDF, 2.28% for ADF, 0.90% for ADL, and 0.27% for CF.

Validations of the metabolism calibrations using the feedlot dataset are reported with the T outliers and the number of samples with $ND > 0.6$ (Table 3). Statistics show estimates with good linearity for OM ($R^2_{val} = 0.80$), excellent for starch ($R^2_{val} > 0.94$), moderate for NDF, and N ($0.70 < R^2_{val} < 0.79$), and poor for ADF, ADL and CF ($R^2_{val} \leq 0.70$). The *SEP* were high for OM, starch, NDF and ADF, indicating poor accuracy of prediction. The slopes deviated substantially from 1 for N, NDF, ADF, and CF with OM and ADF exhibiting the greatest biases (-2.97 and -8.62%, respectively). The number of T outliers was high for all constituents, particularly OM, ADF, and CF. The 10 samples with spiked grain that were added to the feedlot dataset fell into the T outlier group (> 2.5) for all constituents. Aside for ADL, all other nutrients exhibited a high number of samples above the ND threshold, suggesting that additional samples from the feedlot database would strengthen the calibrations. None of the samples displayed a $GD > 5.0$ (data not presented).

The principle component plot (Figure 1a) confirmed that most of the feedlot dataset was within the same population as the digestibility samples, however, there were samples which did not overlap with samples in the digestibility dataset, and outliers (fecal samples spiked with barley grain) were evident. Once the datasets were combined by adding the ND outliers for each constituent, the calibrations displayed similar or improved linearity (R^2) and improved accuracy (lower *SECV*) for all constituents except CF (Table 3).

Calibrations for total tract digestibility

The final calibrations for aTTD of DM, OM, starch and GE yielded moderate to good linearity ($R^2_{CV} \geq 0.71$) between reference and predicted values, and high accuracy ($SECV \leq 2.88\%$; Table 4). Calibrations for aTTD of CP were moderate ($R^2_{CV} \leq 0.62$) and poor for NDF and ADF ($R^2_{CV} \leq 0.33$), with low accuracy as indicated by high $SECV$ values (3.63, 7.86 and 8.83%, respectively).

NIRS validations for total tract digestibility

The validation steps conducted for the NIRS calibrations for aTTD examined the possibility of laboratory bias, but also aimed to describe the influence of certain dietary attributes on predictions. Validations resulted in the removal of 13 samples from one study (Davies et al., 2012) due to laboratory bias as they reduced the predictive statistics for the majority of measured constituents (data not shown). Davies et al. (2012) was the only study where whole shelled or steam rolled corn was fed, and oat hulls replaced barley silage (46-49%). All of the other diets contained barley or corn grain that had been dry- or temper rolled and barley silage as the forage source. Aside from this study, the validations indicated that there was no benefit in developing calibrations that were specific for dry- or temper rolled barley or corn, nor on the basis of the percentage of grain in the diet.

Comparisons between aTTD determined from NIRS predictions using fecal samples collected from feedlot samples and that determined from parallel digestibility studies using total collection are shown in Table 5. The NIRS calibrations over predicted aTTD of DM, starch, N, and NDF, in study 1 (barley or wheat were fed at 89% DM). Aside for aTTD of starch, NIRS was able to predict which diet had a higher or lower digestibility coefficients for DM, CP, NDF, and ADF. For aTTD of starch, NIRS predictions were closer to those derived using Zinn (2002; 2007) equations than those determined from total collection. For the diets fed in study 2, NIRS

predictions of aTTD of DM, OM, starch, CP, and ADF were close to those determined from total collection and the dietary effects on digestibility coefficients identified using total collection were also predicted by NIRS. Fecal NIRS predictions of aTTD of NDF were underpredicted compared to those predicted using total collection. The PCA plot confirmed that samples from both study 1 and study 2 were represented by the calibration set (Figure 1b). The plot also showed that the samples collected from Davies et al. (2012) were outliers within the calibration set.

Discussion

NIRS calibrations for chemical composition of feces

Recently, Landau et al. (2015) developed NIRS calibrations for chemical components of feces using confined beef cows fed 153 forage diets supplemented with barley grain or dried poultry litter, and validated them using samples collected from field studies. They concluded that development of calibrations using confined cattle could be used to predict CP, ash and NDF content of the feces from grazing cattle (Landau et al. 2015). In an earlier study, Coates and Dixon (2011) found that calibrations developed on fecal samples collected from cattle grazing pastures, *in vivo* metabolism trials, and short term pen trials were not reliable predictors of fecal composition across methods. They attributed this to differences in the ranges and distribution of the reference values observed among each method.

In our study, the range in chemical composition between the digestibility and feedlot datasets differed (Table 1), and there were differences noted in their spectral populations (Figure 1a). For most fecal constituents, predictions using the digestibility calibration over- or under-estimated values in the feedlot dataset. These over- or under-estimates resulted in deviations in the slope from 1, and bias from 0. In most cases, the spiked fecal samples were responsible for

these offsets, as they typically deviated the most from the mean of the digestibility samples. This indicated that samples collected from controlled experiments were not representative of the fecal samples with the high levels of starch (>19% of DM) that were collected from penned feedlot cattle (Jancewicz et al. 2016, submitted).

Laboratory bias is a type of error that is difficult to avoid and is encountered when developing NIRS calibrations using multiple labs, technicians, and laboratory methods. Laboratory error can be identified as outliers and eliminated during calibration development and manual validation steps (Coates and Dixon 2011). However, if biases are high, they can impact calibrations and generate poor calibration statistics. To create our database, the scanning of samples from digestibility studies was carried out after wet chemistry analysis. Therefore, it is possible that the chemical composition of samples could have changed during storage. Variation in lab techniques was apparent for ADF, contributing to the large *SEP* and biases observed for this constituent. The two analytical methods used for ADF analysis in our study were sequential (ANKOM) and traditional Van Soest et al. (1991) using filter glass crucibles. Danelón et al. (2012) has reported differences in results obtained using these two methods, with sequential ANKOM ADF values being almost 4% lower for some feeds. When data is collected from multiple laboratories, it can be difficult to ensure that all estimates are generated using exactly the same laboratory procedures.

Combining the metabolism and feedlot datasets dramatically improved the predictive capacity of NIRS for all constituents, except CF. Improvements in *SEP* were noted due to a greater variety of diets being represented in the full dataset, an observation similar to that of Coates and Dixon (2011) when they combined calibration sets based on fecal samples from cattle grazing pastures, *in vivo* digestibility trials, and short term pen trials. Our calibration and cross-

validation statistics for the combined calibrations were comparable to calibrations reported previously for dried ground cattle feces for OM [$R^2_{\text{cal}} = 0.94$ ($SEC = 0.79$) compared to $R^2_{\text{cal}} = 0.94-0.96$ ($SEC = 4.4-1.29$) (Purnomoadi et al. 1996; 1997)], starch [$R^2_{\text{CV}} = 0.96$ ($SECV = 1.67$) compared to $R^2_{\text{CV}} = 0.91$ ($SECV = 0.57$) (Fredin et al. 2014)], N [$R^2_{\text{CV}} = 0.97$ ($SECV = 0.10$) compared to $R^2_{\text{CV}} = 0.96$ ($SECV = 0.08$) (Coates 2004)], NDF [$R^2_{\text{CV}} = 0.92$ ($SECV = 2.42$) compared to $R^2_{\text{CV}} = 0.86$ ($SECV = 1.40$) (Cozzolino et al. 2002)] and ADF [$R^2_{\text{CV}} = 0.92$ ($SECV = 1.97$) compared to $R^2_{\text{CV}} = 0.92$ ($SECV = 1.2$) (Cozzolino et al. 2002)], and ADL [$R^2_{\text{CV}} = 0.92$ ($SECV = 1.00$) compared to $R^2_{\text{CV}} = 0.94$ ($SECV = 1.1$) (Purnomoadi et al. 1996)]. Purnomoadi et al. (1996) also found that calibrations for CF were not predictive for one group of samples. However, other groups provided precise and accurate calibrations for CF (Purnomoadi et al. 1996; 1997). The inability of our study to predict CF likely reflects the narrow range of fat (0.3 - 2.8%) in fecal samples used to develop calibrations.

The official AOAC method for CF used for the samples in our study [Soxtec (991.36)] is vague with regard to extraction time, with any deviations in extraction time altering CF recovery (Palmquist and Jenkins 2003) and increasing the uncertainty of estimates (Hammond 2001). Also, depending if the fat is of dietary or microbial origin, differences in fatty acid composition may cause shifts in wavelength and absorbance, making quantification difficult (Garrido-Varo et al. 2004).

We also found poor validation statistics for ADL in that the R^2_{val} (0.70) was much lower than the R^2_{CV} . This could also reflect the low range in ADL concentration (0.7 - 13%), and the occurrence of samples with low ADL concentration ($\leq 1.0\%$). Accurate measurement of ADL using NIRS enabled previous researchers to use ADL as a digestibility marker (Purnomoadi et al. 1996, 1997; Jancewicz et al. 2016). In our recent study, ADL analysis was performed by one

technician, and the coefficient of variation between replicates was below 2% ensuring excellent precision. Care must be taken when using NIRS predicted ADL for digestibility estimates since errors will be amplified, especially since ADL concentrations are typically low in high-grain feedlot diets. Our calibration statistics compare favourably with previously published reports for OM, starch, N, NDF, and ADF and can be implemented for quantitative measurement of these fecal nutrients in commercial feedlots that feed diets similar to those represented in the calibration.

NIRS calibrations for total tract digestibility

To our knowledge, there are only three studies where NIRS calibrations were developed to predict nutrient digestibility based on total collection of feces from cattle that had been fed forage and concentrate diets. Of these three, two developed fecal NIRS calibrations for predicting aTTD of OM (de la Roza et al. 2002) and DM (Garnsworthy and Unal 2004) in dairy cattle and one estimated the aTTD of DM in beef steers (Gibbs et al. 2002). Our results are comparable to those obtained in these studies for aTTD of DM [$R^2_{\text{cal}} = 0.86$, $SECV = 2.9$ compared to $R^2_{\text{cal}} = 0.87$, $SECV = 3.0$ (Gibbs et al. 2002)] and OM [$R^2_{\text{cal}} = 0.88$, $SECV = 2.9$ compared to $R^2_{\text{cal}} = 0.86$, $SECV = 2.6$ (de la Roza et al. 2002)].

The calibrations developed in our study were used in a concurrent study that examined the variation in fecal excretion and digestibility estimates over 24 h, with the calibrations for predicting aTTD of starch being closer to estimates derived by total collection than all other nutrients (Jancewicz et al. 2016). It is not surprising that NIRS is more accurate at predicting aTTD of starch than other fecal constituents, given that other studies have documented the close relationship between fecal starch and starch digestibility in high-grain diets (Zinn et al. 2002, 2007; Fredin et al. 2014). The same calibrations for aTTD of starch were not as predictive when

grain comprised only 44% of the diet DM as less starch in the diet resulted in less starch in the feces.

Using fecal NIRS to predict digestibility of other nutrients is more difficult than predicting chemical composition directly, explaining the poorer calibration statistics associated with these estimates. Using NIRS for predicting aTTD measures the absorption of the nutrient peaks arising from the feces, and not all changes in fecal composition reflect changes in digestibility. For example, endogenous secretions contain N, which is excreted to a greater extent with more fermentable diets (Oba and Allen 2003). Many of the diets used in calibration development contained by-products including dried distillers' grains with solubles, grains screenings and added oil, which likely increased fecal concentrations of N and CF without directly affecting digestibility. Altering the dietary proportions of forage and grain in the diet would be expected to induce greater variation in the chemical composition of fecal samples and thereby lead to more robust calibrations (Garnsworthy and Unal 2004). The particularly poor predictions of aTTD of NDF and ADF could also likely be due to their relatively low digestibility compared to the other nutrients. Lower digestibility would result in larger coefficients of variation, reducing the predictive ability of aTTD of NDF and ADF relative to starch (Fredin et al. 2014).

Unlike for chemical composition, validation methods for calibrations for aTTD are impractical as they require performing time consuming and costly digestibility studies. To gauge how closely our predictions of aTTD of DM, OM, starch, CP, NDF, and ADF in the feedlot studies were to results from total collection, we compared predictions to aTTD determined in two parallel digestibility studies (He et al. 2015; Chibisa et al. unpublished). Ideally a greater number of studies would be more informative with regard to the robustness of calibrations.

Predictions for study 2 were much more accurate than study 1 and this is likely because the diets from study 2 were included in the calibration set (Figure 1b). Study 1 included wheat in one of the diets, and samples from cattle fed wheat were not included in the calibration set.

The NIRS predicted starch digestibility in study 1 was much closer to starch digestibility estimates generated using the equations of Zinn et al. (2002; 2007; Table 5), providing evidence that digestibility may have differed between parallel digestibility and feedlot studies. Similar to our predictions, Koenig and Beauchemin (2011) also failed to find differences in estimated starch digestibility when varying proportions of barley silage was fed to feedlot cattle, a result confirmed in study 2 (Chibisa et al. unpublished).

Landau et al. (2015) and Coates and Dixon (2011) found that predicting *in vitro* DM and OM digestibility was possible if the range in digestibility values encompassed those in the validation sets. However, when considering NIRS aTTD predictions, differences in DMI may affect aTTD. Competition is known to alter intake, which can in turn affect digestibility. Predicting DMI using calibrations developed for confined cattle has not always been successful as many dietary and physiological factors affect intake, particularly of forage diets (Dixon and Coates. 2009; Landeau et al. 2015). Intakes for barley and wheat fed steers were greater in study 1 (He et al. 2015) compared to cattle in our feedlot study, and lower in study 2 (Chibisa et al. unpublished). These differences could have led to discrepancies between direct and NIRS predicted estimates of aTTD. Additional discrepancies such as differences in grain processing between feedlot and digestibility studies may have also resulted in the deviations observed.

Calibrations for aTTD of nutrients developed using cattle in metabolism experiments shows potential for predicting samples collected from feedlot cattle, but calibrations are more predictive when the diets are represented in the calibration set. By continuing to expand the

calibration by adding more samples, predictability of aTTD could be made applicable to a wider range of diets fed to feedlot cattle. In addition, PCA can be used as an additional technique to rapidly assess whether unknown samples fit within a calibration population.

Conclusion and implications

Fecal NIRS calibrations can be applied for use in commercial feedlots for accurate quantitative predictions of fecal chemical composition. Fecal starch in particular, has been used to predict starch digestibility and proper grain processing, which may be used to make management decisions that improve feed efficiency. However, consistent reference analysis is crucial for developing accurate calibrations. Calibrations for fecal CF proved to lack precision owing to the low CF concentration in the diets and consequently in the feces. Calibration for aTTD of nutrients can be used for qualitative tracking of digestibility when the diet or grain processing is changed. Additional diets with differing composition should be added to the original database so as to further increase the accuracy of future predictions of those diets that are employed in the Canadian feedlot industry.

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Table 1. Dietary ingredient composition and range in fecal chemical composition of organic matter (OM), starch, nitrogen (N), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and crude fat (CF) from digestibility and feedlot studies with cattle whose fecal samples were used for development of fecal near infrared spectroscopy calibrations.

Dietary ingredients : Grain, Forage, other ^{a,b} (% of diet DM)	n (nd)	Range in composition (% fecal DM)						
		OM	Starch	N	NDF	ADF	ADL	CF
<i>Digestibility Dataset</i>								
DRC (80), barley silage (10), none	19(1)	88-93	4.3-24	2.4-3.5	30-47	15-24	-	-
DRB (0, 35), barley silage (55), DDGS (37.6, 40)	32(4)	83-89	-	2.4-3.5	49-63	38-44	-	-
DRB (47, 87), barley silage (8), DDGS (37.6, 40)	32(4)	84-90	0.7-14	2.4-3.5	42-58	22-31	-	-
WSC/SRC (0, 43), none, oat hulls (46-49)	13(4)	89-92	0.3-5.0	0.8-1.4	69-82	32-40	-	0.9-2.5
TRB (43-86), barley silage (9), DDGS (20, 40)	137(4)	83-93	3.6-28	2.1-3.9	39-57	19-35	-	1.4-2.4
DRB (44), barley silage (44), none	144(1)	81-85	0.7-4.0	2.3-2.9	55-60	37-40	11-12	1.2-2.8
DRB (80), barley silage (9), none	144(1)	86-90	2.4-11	2.6-3.1	46-61	25-33	7.4-10	1.8-2.7
DRB (68-80), barley silage (0-12), DDGS (15)	32(4)	87-91	2.4-10	2.5-3.5	46-57	18-25	-	-
DRB (0-32), barley silage (36), grass hay (24), wheat bran (0-32)	24(4)	-	-	1.8-2.1	56-67	37-42	-	-
DRB (47-87), barley silage (8), DDGS (20-40)	24(4)	-	-	2.3-4.2	-	-	-	-
<i>Feedlot Dataset</i>								
DRB (56-76), barley silage (9), GSP(0, 20) DDGS(10)	42(3)	82-90	3.7-12	2.2-2.8	47-58	25-34	4.3-8.1	1.3-2.4
DRB (57-87), barley silage (10), DDGS (0-30)	18(4)	-	2.7-12	1.8-2.9	54-65	37-48	-	-
DRB/DRW (89), barley silage (6), none	92(2)	65-85	3.5-33	2.2-2.5	31-56	15-33	4.3-7.7	0.9-2.1
DRB (68-80), barley silage (0-12), DDGS (15)	8 (4)	72-90	3.6-12	2.2-2.5	49-56	24-32	3.7-7.2	0.3-1.8
DRB, variety (7-27), variety	20(4)	88-96	19-53	1.9-2.4	22-41	8.5-22	0.7-3.1	1.5-2.2
DRB/TRB/DRW/TRW variety (7-27), variety	288(24)	70-88	2.1-24	2.0-2.4	22-38	33-61	3.8-5.4	0.4-0.8
none or DRB ^y , variety (55-100), variety	41(5)	77-87	0.2-3.5	1.9-2.6	55-63	39-49	10-13	1.3-2.1

Abbreviations: DRC = dry rolled corn; DRB = dry rolled barely; DDGS = dried distillers grains with solubles (wheat or corn); WSC = whole shelled corn; SRC = steam rolled corn; TRB = temper rolled barley; DRW = dry rolled wheat; TRW = temper rolled wheat; GSP = grain screening pellets; variety = indicates samples obtained from cattle fed a variety of different diets and their type and proportions are not listed; n=number of samples; nd= number of diets of different chemical composition.

^aList of references in descending order; Digestibility dataset: Vyas et al. 2014; Hunerberg et al. 2013a; Hunerberg et al. 2013b; Davies et al. 2012; Koenig et al, unpublished; Jancewicz et al. 2016; Jancewicz et al. 2016; Chibisa et al. unpublished; Friedt et al. 2014; McKinnon and Walker 2008; Feedlot dataset: Jancewicz et al. unpublished; He et al. 2015; Moya et al. 2015; Koenig et al unpublished; Jancewicz et al. unpublished; Jancewicz et al. unpublished; Jancewicz et al. unpublished.

^bDoes not include feed additives or vitamin and mineral or other supplement.

Table 2. Dietary ingredient composition and range in digestibility of dry matter (DM), organic matter (OM), starch, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and gross energy (GE) from digestibility studies with cattle whose fecal samples were used for development of fecal near infrared spectroscopy calibrations and parallel feedlot studies used for evaluation of calibrations.

Dietary ingredients : Grain, Forage, other ^{a,b} (% of diet DM)	n (nd)	Range in apparent total tract digestibility (% of intake)						
		DM	OM	Starch	CP	NDF	ADF	GE
<i>Digestibility dataset</i>								
DRC (80), barley silage (10), none	19(1)	77-80	78-81	93-96	61-75	41-46	39-43	75-78
DRB (0, 35), barley silage (55), DDGS (37.6, 40)	32(4)	66-71	66-72	-	61-75	44-51	32-43	66-70
DRB (47, 87), barley silage (8), DDGS (37.6, 40)	32(4)	73-82	75-84	-	71-85	49-55	36-57	74-82
WS/SR C (0, 43), none, oat hulls (46-49)	13(4)	66-71	68-72	98-99	-	41-50	41-50	n/a
TRB (43-86), barley silage (9), DDGS (20, 40)	32(4)	67-84	69-85	83-98	66-84	38-71	6.1-63	67-84
DRB (44), barley silage (44), none	6(1)	73-78	76-79	98-99	-	50-57	40-49	74-78
DRB (80), barley silage (9), none	6(1)	76-82	81-87	94-98	-	63-71	44-52	76-82
DRB (68-80), barley silage (0-12), DDGS (15)	32(4)	71-90	72-91	94-100	63-87	34-76	8.5-68	-
<i>Validation studies</i>								
DRB/DRW (89), barley silage (6), none (Study 1)	8(2)	70-73	-	92-94	59-64	49-52	38-39	-
DRB (68-80), barley silage (0-12), DDGS (15) (Study 2)	20(4)	79-86	81-87	97-99	76-82	59-69	40-54	-

Abbreviations: DRC = dry rolled corn; DRB = dry rolled barely; DDGS = dried distillers grains with solubles (wheat or corn); WSC = whole shelled corn; SRC = steam rolled corn; TRB = temper rolled barley; DRW = dry rolled wheat; TRW = temper rolled wheat; GSP = grain screening pellets; variety = indicates samples obtained from cattle fed a variety of different diets and their type and proportions are not listed; n=number of samples; nd= number of diets of different chemical composition.

^aList of references in descending order; Digestibility dataset: Vyas et al. 2014; Hunerberg et al. 2013a; Hunerberg et al. 2013b; Davies et al. 2012; Koenig et al, unpublished; Jancewicz et al. 2016; Jancewicz et al. 2016; Chibisa et al. unpublished; Validation dataset: Moya et al. 2015; Koenig et al unpublished.

^bDoes not include feed additives or vitamin and mineral or other supplement.

Table 3. Statistics for near infrared spectroscopy calibrations determined by partial least squares regression for fecal constituents including organic matter (OM), starch, nitrogen, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and crude fat (CF) using the digestibility dataset validated with the feedlot dataset and the combined datasets.

Item	Calibration Statistics ^a							Validation Statistics with Feedlot dataset							
	n	Outliers	Range (Mean)	R^2_{cal}	SEC	R^2_{CV}	$SECV$	n	Range (Mean)	R^2_{val}	SEP	slope	bias	T>2.5	ND>0.6
OM															
Digestibility dataset	244	4	77.5-93.4 (89.2)	0.89	0.81	0.83	0.96	53	69.6-96.4 (85.4)	0.80	3.67	1.03	-2.97	38	42
Combined datasets	283	5	69.6-96.4 (88.5)	0.94	0.79	0.90	1.02								
Starch															
Digestibility dataset	149	7	0.2-24.3 (6.3)	0.93	1.18	0.87	1.38	53	0.7-53.0 (16.0)	0.94	4.00	1.28	1.03	18	28
Combined datasets	197	6	0.2-51.7 (7.8)	0.97	1.49	0.96	1.67								
Nitrogen															
Digestibility dataset	208	0	0.8-4.2 (2.6)	0.98	0.093	0.97	0.108	49	1.8-2.8 (2.3)	0.75	0.24	0.62	-0.16	14	46
Combined datasets	245	1	0.8-4.2 (2.5)	0.98	0.090	0.97	0.100								
NDF															
Digestibility dataset	326	4	30.4-81.5 (51.9)	0.92	2.21	0.90	2.41	49	19.7-61.2 (45.3)	0.73	6.85	1.35	-0.18	19	39
Combined datasets	347	2	19.7-81.5 (51.8)	0.95	1.90	0.92	2.42								
ADF															
Digestibility dataset	179	1	4.9-48.5 (29.2)	0.97	1.53	0.93	2.28	48	8.5-37.8 (25.6)	0.25	11.0	0.71	-8.62	20	44
Combined datasets	225	0	4.9-48.5 (28.4)	0.94	1.85	0.92	1.97								
ADL															
Digestibility dataset	26	0	3.1-13.0 (10.0)	1.00	0.07	0.85	0.90	45	0.70-8.0 (4.9)	0.70	1.57	1.06	-1.00	12	3
Combined datasets	55	0	0.7-13.0 (7.1)	0.95	0.83	0.92	1.00								
CF															
Digestibility dataset	40	2	0.9-2.8 (1.8)	0.70	0.23	0.43	0.27	45	0.3-2.4 (1.5)	0.25	2.82	0.48	-1.96	23	45
Combined datasets	78	9	0.9-2.8 (1.7)	0.53	0.28	0.40	0.32								

Abbreviations: R^2_{cal} = coefficient of determination of calibration; SEC = standard error of calibration; R^2_{CV} = coefficient of determination of cross-validation; $SECV$ = standard error of cross validation; R^2_{val} = coefficient of determination of validation; SEP = standard error of performance; T = T limit; ND = neighbourhood distance.

^aMath treatments 1, 8, 8, 1, standard normal variate and detrend. Spectra were clipped between 1250-2350 nm for all constituents except for crude fat (1200-2400 nm).

Table 4. Statistics for near infrared spectroscopy calibrations determined by partial least squares regression for apparent total tract digestibility of dry matter, (DM), organic matter (OM), starch, nitrogen, neutral detergent fiber (NDF), acid detergent fiber (ADF), and gross energy (GE).

Item	Calibration Statistics ^a						
	n	Outliers	Range (Mean)	R^2_{cal}	SEC	R^2_{CV}	SECV
<i>Digestibility, % of intake</i>							
DM	156	2	59.4-90.1 (76.5)	0.86	2.18	0.75	2.88
OM	156	2	57.4-91.0 (77.8)	0.88	2.18	0.77	2.88
Starch	92	2	87.2-99.4 (95.9)	0.93	0.74	0.84	1.06
Crude Protein	144	2	61.1-86.7 (73.9)	0.73	3.13	0.62	3.63
NDF	154	4	26.4-76.2 (54.3)	0.48	7.10	0.33	7.86
ADF	153	5	6.1-71.9 (43.9)	0.46	7.25	0.21	8.83
GE	124	2	57.4-88.7 (74.8)	0.81	2.34	0.71	2.79

Abbreviations: R^2_{cal} = coefficient of determination of calibration; SEC = standard error of calibration; R^2_{CV} = coefficient of determination of cross-validation; SECV = standard error of cross validation; ^aMath treatments 1, 8, 8, 1, SNV and detrend. Spectra were clipped between 1250-2350 nm.

Table 5: Digestibility values predicted using NIRS equations in feedlot studies where cattle were fed barley or wheat in study 1 or barley and increasing levels of silage in study 2, as compared to estimates (Actual) derived from total collection in digestibility studies.

Digestibility Parameter	Study 1			Study 2				
	B79	W80	SEM	SL0	SL4	SL8	SL12	SEM
Dry Matter								
Feedlot study (NIRS)	79.7	80.1	0.76	82.4 _a	81.0 _b	79.7 _c	78.8 _c	0.357
Digestibility study (Actual)	70.0	73.0	1.26	86.0 _a	82.1 _b	81.1 _{bc}	79.5 _c	0.691
<i>P</i> value	<0.01	<0.01		<0.01	0.15	0.08	0.35	
Organic Matter								
Feedlot study (NIRS)	82.0	82.8	0.61	85.1 _a	83.1 _b	81.5 _c	80.5 _c	0.39
Digestibility study (Actual)	NM	NM	-	87.2 _a	83.7 _b	82.4 _{bc}	81.1 _c	0.75
<i>P</i> value	-	-		0.01	0.48	0.25	0.46	
Starch								
Feedlot study (NIRS)	95.3	94.3	0.42	97.5	97.5	97.7	97.8	0.62
Digestibility study (Actual)	91.9	93.1	0.70	98.9 _a	98.0 _b	97.9 _b	96.8 _c	0.32
<i>P</i> value	<0.01	0.14		0.05	0.54	0.75	0.16	
Feedlot study (NIRS)	95.2	94.1	0.67	97.5	97.5	97.7	97.8	0.42
Zinn et al. 2002 ^a	96.0	93.7		96.3	96.3	96.5	96.6	
<i>P</i> value	0.23	0.68		<0.01	<0.01	<0.01	<0.01	
Feedlot study (NIRS)	95.2	94.1	0.59	97.5	97.5	97.7	97.8	0.40
Zinn et al. 2007 ^a	96.2	95.2		96.7	96.4	96.4	96.5	
<i>P</i> value	0.13	0.16		0.06	<0.01	<0.01	<0.01	
Crude Protein								
Feedlot study (NIRS)	74.4	74.8	0.85	79.7 _a	78.4 _a	76.5 _b	75.0 _b	0.58
Digestibility study (Actual)	63.9 _a	59.1 _b	1.41	81.7 _a	76.8 _{ab}	77.3 _{ab}	75.9 _b	1.12
<i>P</i> value	<0.01	<0.01		0.12	0.18	0.56	0.50	
NDF								
Feedlot study (NIRS)	57.2	56.4	1.41	59.8 _a	57.0 _b	54.7 _c	52.9 _c	0.82
Digestibility study (Actual)	51.6	49.5	2.33	68.9 _a	59.5 _b	59.3 _b	58.9 _b	1.58
<i>P</i> value	0.04	0.01		<0.01	0.16	0.01	<0.01	
ADF								
Feedlot study (NIRS)	43.9	41.7	1.87	51.9 _a	49.4 _{ab}	46.7 _{bc}	44.5 _c	1.11
Digestibility study (Actual)	39.3	37.7	3.11	53.5 _a	40.2 _b	41.9 _b	44.3 _b	2.16
<i>P</i> value	0.21	0.26		0.65	<0.01	0.05	0.93	

Note: Italicized letters in each row for each study represent significant differences ($P < 0.05$).

Abbreviations: B79 = barley processed to a PI of 79% where PI is processing index calculated as ratio of the bushel weight after processing over the bushel weight before processing; W80 = wheat processed to 80%; SL = silage level; NIRS = digestibility determined using near infrared spectroscopy calibrations; NM = not measured; NDF = neutral detergent fiber; ADF = acid detergent fiber.

^aEquations derived by Zinn et al (2002, 2007) to estimated total tract starch digestibility for feedlot samples using NIRS predicted fecal composition.

Figure 1 : Principle component plot showing the two populations of spectra; (a) the feedlot and digestibility (PEN) datasets for the NIRS calibrations for fecal chemical composition, and (b) the metabolism (MET) dataset for the calibrations for apparent total tract digestibility, and the two external validations for Study 1 (ST1) and 2 (ST2).