*Translational Animal Science*, 2023, **7**, txad043
https://doi.org/10.1093/tas/txad043
Advance access publication 2 May 2023
**Ruminant Nutrition**

# Predicting fecal composition, intake, and nutrient digestibility in beef cattle consuming high forage diets using near infrared spectroscopy

**Jenilee F. Peters,[†,‡] Mary L. Swift,[‡] Gregory B. Penner,[†] Herbert A. Lardner,[†] Tim A. McAllister,[†,‖] and Gabriel O. Ribeiro[†,1]**

[†]Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5A8
[‡]Trouw Nutrition Canada, Okotoks, Alberta, Canada T1S 1A2
[‖]Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada T1J 4B1
[1]Corresponding author: gabriel.ribeiro@usask.ca

## Abstract

The objective of this study was to develop near infrared spectroscopy (NIRS) calibrations to predict fecal nutrient composition, intake, and diet digestibility from beef cattle fed high forage diets. Heifers were fed 12 different forage-based diets (>95% forage dry matter basis) in 3 total collection digestibility studies, resulting in individual fecal samples and related spectra ($n$ = 135), corresponding nutrient intake, and apparent total tract digestibility (aTTD) data. Fecal samples were also collected from steers grazing two annual and two perennial forage mixtures over two growing seasons. Samples ($n$ = 13/paddock) were composited by paddock resulting in 30 samples from year 1, and 24 from year 2. The grazing fecal spectra ($n$ = 54) were added to the existing fecal composition spectral library. Dried and ground fecal samples were scanned using a FOSS DS2500 scanning monochromator (FOSS, Eden Prairie, MN). Spectra were mathematically treated for detrend and scatter correction and modified partial least squares (MPLS) regression was performed. The coefficient of determination for cross validation ($R^2_{cv}$) and standard error of cross validation (SECV) were used to evaluate the quality of calibrations. Prediction equations were developed for fecal composition [organic matter (OM), nitrogen (N), amylase-treated ash-corrected neutral detergent fiber (aNDFom), acid detergent fiber (ADF), acid detergent lignin (ADL), undigestible NDF after 240 h of in vitro incubation (uNDF), calcium (Ca), and phosphorus (P)], digestibility [DM, OM, aNDFom, N], and intake [DM, OM, aNDFom, N, uNDF]. The calibrations for fecal OM, N, aNDFom, ADF, ADL, uNDF, Ca, P resulted in $R^2_{cv}$ between 0.86 and 0.97 and SECV of 1.88, 0.07, 1.70, 1.10, 0.61, 2.00, 0.18, and 0.06, respectively. Equations predicting intake of DM, OM, N, aNDFom, ADL, and uNDF resulted in $R^2_{cv}$ values between 0.59 and 0.91, SECV values of 1.12, 1.10, 0.02, 0.69, 0.06, 0.24 kg·d$^{-1}$, respectively, and SECV values between 0.00 and 0.16 when expressed as % body weight (BW). Digestibility calibrations for DM, OM, aNDFom, and N resulted in $R^2_{cv}$ ranging from 0.65 to 0.74 and SECV values from 2.20 to 2.82. We confirm the potential of NIRS to predict fecal chemical composition, digestibility, and intake of cattle fed high forage diets. Future steps include validation of the intake calibration equations for grazing cattle using forage internal marker and modelling energetics of grazing growth performance.

## Lay Summary

Efficient and sustainable management of grazing production systems requires real-time, easy, and cost-effective nutritional analysis of forage quality. Wet chemistry analysis is costly and time-consuming whereas near infrared spectroscopy (NIRS) can provide rapid, cost-effective, and accurate predictions of nutrient composition, and estimates of intake and digestibility. This study determined the potential of NIRS scanning of the feces to predict fecal composition, intake, and diet digestibility of beef cattle consuming high forage diets. Heifers were fed forage diets (> 95% forage dry matter basis) in three total collection digestibility studies in which fecal samples were collected, feed intake was measured, and apparent total tract digestibility (aTTD) was determined. Fecal samples were also collected from steers grazing four forage mixtures over two growing seasons. Fecal spectra were collected and regression equations of moderate to excellent quality for chemical composition, nutrient intake, and digestibility were developed. We confirm the potential of NIRS to predict fecal chemical composition, as well as digestibility and intake of cattle fed high forage diets.

**Key words:** digestibility, feces, fecal composition, grazing cattle, intake, near infrared spectroscopy

## INTRODUCTION

Dynamic beef cattle grazing systems require effective management strategies to maintain profitability. Improving feed efficiency of grazing beef cattle can increase the productivity and sustainability of cow-calf and pasture-based backgrounding systems (Basarab et al., 2013). However, there is a need for simple, cost-effective, and accurate analyses to assess forage quality and production, manage grazing strategies, and respond to rapidly changing pasture conditions.

The intake and digestibility of forages and pastures is largely influenced by the fiber content (NDF) and its degree of lignification (Harper and McNeill, 2015). The composition and structure of NDF influences feeding and rumination

Received March 15, 2023 Accepted April 27, 2023.

**Table 1.** Range [min-max(mean)] in fecal composition of samples used for the development of near infrared spectroscopy calibrations.

| | Study 1 | Study 2 | Study 3 | Grazing Year 1 | Grazing Year 2 |
|---|---|---|---|---|---|
| *n* | 32 | 32 | 72 | 30 | 24 |
| Fecal composition[1] (% DM) | | | | | |
| OM | 81.4-91.1 (86.4) | 81.3-87.9 (84.9) | 86.4-90.4 (88.6) | 53.4-79.0 (68.6) | 49.7-78.5 (64.9) |
| N | 1.70-2.42 (2.12) | 2.00-2.48 (2.21) | 1.55-2.32 (1.86) | 1.24-2.27 (1.70) | 1.34-2.14 (1.63) |
| aNDFom | 49.9-66.8 (56.9) | 48.8-59.9 (54.3) | 52.8-62.4 (59.6) | 30.8-58.2 (44.7) | 25.8-64.5 (48.8) |
| ADF | 41.3-50.7 (46.3) | 41.9-49.5 (45.0) | 34.0-48.3 (42.9) | 26.3-53.1 (43.5) | 31.7-58.7 (49.3) |
| ADL | 15.6-20.2 (18.0) | 15.4-19.0 (17.1) | 8.20-16.0 (13.1) | 6.35-16.3 (12.0) | 7.54-17.5 (14.0) |
| uNDF | 36.3-55.4 (45.1) | 36.8-49.1 (42.6) | 29.8-49.3 (42.6) | 21.0-46.6 (31.4) | 21.6-50.3 (35.1) |
| Ca | 1.30-4.54 (3.02) | 2.43-3.51 (2.97) | 0.70-1.67 (1.32) | 0.52-6.86 (2.19) | 0.45-4.04 (1.36) |
| P | 0.31-0.92 (0.58) | 0.33-0.78 (0.61) | 0.36-0.97 (0.54) | 0.30-1.27 (0.69) | 0.20-0.78 (0.32) |

[1]DM = dry matter; OM = organic matter, N = nitrogen, aNDFom = amylase-treated ash-corrected neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin, uNDF = undigestible amylase-treated ash-corrected NDF after 240 h of in vitro incubation, Ca = calcium, P = phosphorus.

behavior, rate of particle breakdown, ruminal turnover and fill, and consequently dry matter intake (DMI) and average daily gain (ADG) of cattle (Harper and McNeill, 2015). Undigested NDF (uNDF) is the fraction of NDF not available for microbial digestion in ruminants, even if its residency in the total tract is infinite (Huhtanen et al., 2007). As cattle intake in grazing systems is often controlled by rumen fill and the rate of disappearance of ingesta (Ellis, 1978), a higher uNDF intake may limit forage intake. The use of uNDF content of forages and diets as an indicator of intake and digestibility has received increasing interest, and nutritional models have included it as an important factor in predicting feed intake and digestibility (Lippke et al., 1986; van Amburgh et al., 2015).

Forage grazing management decisions often begin with identifying pasture yield and nutrient composition and augmenting this information with estimates for intake and digestibly would provide value. Collecting and determining intake and digestibility of grazing forages is a challenge, often relying on total collection confinement studies or techniques that rely on external or internal markers (Decruyenaere et al., 2009). Near infrared spectroscopy (NIRS), and specifically NIRS of feces, has been identified as a rapid and accurate alternative for predicting intake and digestibility (Dixon and Coates, 2009; Jancewicz, et al., 2017a, 2017b; Johnson et al., 2017). However, the need to develop large reference databases that require frequent updating to maintain robust calibrations that account for variation in forage composition over diverse growing conditions limits the practical application of NIRS. Brogna et al. (2018) demonstrated that NIRS could be used to accurately predict fecal uNDF from dairy cows fed a total mixed ration. The objective of this study was to develop near infrared spectroscopy calibrations to predict fecal nutrient composition including uNDF and lignin, and intake and digestibility from beef cattle fed forage diets.

## MATERIALS AND METHODS

All animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (Ottawa, ON, Canada), and animal use was reviewed and approved by the University of Saskatchewan Animal Research Ethics Board (Protocols #20200046, 20200079, 20090107)

### Origin of Samples

A library of fecal samples for the development of NIRS calibrations for the prediction of fecal composition (Table 1), intake, and aTTD (Table 2) was compiled from cattle fed high forage diets in three digestibility studies and from beef steers grazing a variety of pastures species over two consecutive years.

### Digestibility Studies 1 and 2

Two digestibility studies were conducted at the University of Saskatchewan Livestock and Forage Center of Excellence (LFCE) Saskatchewan Cattleman's Association metabolism barn (Clavet, SK, Canada).

*Experimental design* Experiments were designed as a replicated 4 × 4 Latin square with eight heifers (four/square) with four 28 d periods and four dietary treatments per study. Heifers were adapted to each diet for the first 14 d of each period, DMI was measured from days 15 to 28, and fecal samples were collected from days 25 to 28 to estimate aTTD.

Eight commercial Angus × Hereford crossbred heifers (298 ± 18 kg initial body weight (BW); 456 ± 27 kg end BW) were randomly assigned to two groups, with four heifers in each group (i.e., squares 1 and 2), and housed in tie stalls. Heifers were allowed daily exercise for 2 h/d in an open dry lot, except during the total collection periods or when temperatures dropped below −20 °C. Heifers were offered the treatment diet ad libitum, once daily at 0930 h, targeting a minimum of 10% refusals. Prior to feeding, hay was chopped using a commercial tub-style grinder (Highline Manufacturing Model CFR1251, Vonda, SK, Canada) with a 5 cm primary screen. Particle size of the chopped forages was determined using the Penn State Particle Separator method as described by Kononoff et al. (2003). Diets of the first study included alfalfa and timothy hay harvested at different maturities, described as early and late maturity. Varieties grown comprised of 1) alfalfa common #1 (*Medicago sativa* L.) for both early and late treatments; 2) Richmond timothy (*Phleum pratense*) for the early treatment; and 3) Climax timothy (*Phleum pratense*) for the late treatment. The timothy and alfalfa forages were grown in the dark brown soil zone near Outlook, Saskatchewan, Canada (long 51°30′N, lat 10703'W. All forages were grown in the same production year (2020) with forage stands being less than 3 years old.

**Table 2.** Range [min-max(mean)] in intake of nutrients of samples used for the development of near infrared spectroscopy calibrations.

| | Study 1 | Study 2 | Study 3 |
|---|---|---|---|
| *n* | 32 | 32 | 72 |
| Intake[1], kg·d$^{-1}$ | | | |
| DM | 3.91-11.2 (7.71) | 5.92-11.5 (9.34) | 8.83-16.9 (13.0) |
| OM | 3.47-9.97 (6.96) | 5.37-10.6 (8.47) | 8.23-15.8 (12.1) |
| N | 0.08-0.34 (0.19) | 0.14-0.30 (0.22) | 0.17-0.31 (0.24) |
| aNDFom | 2.27-6.32 (4.00) | 3.34-6.19 (5.04) | 4.83-8.88 (6.90) |
| ADL | 0.29-0.74 (0.53) | 0.39-0.71 (0.59) | 0.41-0.79 (0.62) |
| uNDF | 0.91-2.30 (1.64) | 1.37-2.41 (1.88) | 1.27-2.84 (2.13) |
| % BW | | | |
| DM | 1.43-3.00 (2.30) | 1.75-2.79 (2.21) | 1.36-2.65 (2.04) |
| OM | 1.25-2.63 (2.08) | 1.61-2.53 (2.00) | 1.27-2.48 (1.91) |
| N | 0.03-0.09 (0.06) | 0.03-0.07 (0.05) | 0.03-0.05 (0.04) |
| aNDFom | 0.70-1.67 (1.19) | 0.93-1.50 (1.19) | 0.78-1.42 (1.08) |
| ADL | 0.11-0.22 (0.16) | 0.11-0.16 (0.14) | 0.06-0.12 (0.10) |
| uNDF | 0.34-0.67 (0.49) | 0.32-0.55 (0.45) | 0.20-0.45 (0.33 |
| g·kg$^{-1}$ BW$^{0.75}$ | | | |
| DM | 59.2-131 (98.4) | 75.6-126 (100) | 68.7-133 (103) |
| OM | 52.7-116 (88.7) | 68.7-114 (90.6) | 64.0-124 (95.8) |
| N | 1.20-3.96 (2.41) | 1.57-3.29 (2.39) | 1.35-2.44 (1.91) |
| aNDFom | 31.3-73.6 (50.8) | 42.7-67.6 (54.0) | 39.1-70.0 (54.3) |
| ADL | 4.45-9.32 (6.72) | 4.97-7.36 (6.28) | 3.24-5.99 (4.86) |
| uNDF, | 13.9-28.9 (20.9) | 15.0-25.1 (20.2) | 10.3-22.1 (16.8) |
| Digestibility, % | | | |
| DM | 52.1-70.4 (62.8) | 53.5-68.0 (59.6) | 55.6-74.3 (64.7) |
| OM | 55.2-72.2 (64.4) | 55.5-69.5 (62.2) | 57.9-75.7 (66.5) |
| N | 38.6-77.7 (65.9) | 45.2-71.3 (62.0) | 57.5-72.8 (65.1) |
| aNDFom | 49.4-71.3 (58.6) | 50.9-67.0 (59.4) | 45.0-66.1 (60.5) |

[1]DM = dry matter, OM = organic matter, N = nitrogen, aNDFom = amylase-treated ash-corrected neutral detergent fiber, ADL = acid detergent lignin, uNDF = undigested amylase-treated ash-corrected NDF after 240 h of in vitro incubation.

After soil analysis, timothy forages were broadcast fertilized at the beginning of the growing season (mid-May) with 45.4, 13.6, 18.1, and 6.4 kg ha$^{-1}$ of nitrogen (N), phosphorus (P), potassium (K), and sulfur (S), respectively. The alfalfa forages were broadcast fertilized at the same time with 1.1, 5.5, and 18.4 kg ha$^{-1}$ of N, P, and K, respectively. Precipitation data were obtained from Environment Canada's Climate Data for Outlook, Saskatchewan, Canada (long 5130'N, lat 107°03'W, www.climate.weather.gc.ca). Total precipitation of the growing area for April, May, June, and the first 10 d of July (up to final cutting date) was 10.5, 30.1, 92.3, 19.8 mm, respectively. Additionally, the early timothy received 5 cm of water by irrigation up to cutting, with the other forages receiving 7.5 cm each. The early timothy hay was harvested at early head, followed by the late hay harvested at the flowering stage as described by Moore and Moser (1995). The early alfalfa was harvested at 10% bloom, and the late alfalfa at full flower as described by Kalu and Fick (1981). In the second study, the same four forages were blended 50:50 resulting in treatment diets of early alfalfa × early timothy (EAET), early alfalfa × late timothy (EALT); late alfalfa × early timothy (LAET); and late alfalfa × late timothy. A free-choice mineral-vitamin supplement (Hi-Range Beef Summer Mineral, Trouw Nutrition, Guelph, ON, Canada) and fresh water were provided ad libitum during both studies.

For both studies, the weights (as is) of forage offered and refused were recorded daily but only data used from d 18 to 25 were used to calculate individual feed intake. Samples of forages were taken on an equal proportion basis (as is) daily and composited by week. Feed refusals were collected and weighed daily during the total collection period (d 25 to 28), mixed, and subsampled (5% wet weight) to obtain a composited sample. During fecal collection, feces were collected by placing pans behind the heifers and pens were scraped hourly between 700 h and 1800 h. To prevent urine contamination of the feces, indwelling catheters (26 French, 75-cc ballon; C. R. Bard, Inc., Covington, GA) were inserted into the bladder of each heifer. The individual total quantity of feces excreted was recorded daily, and a representative sample (10% wet weight) was collected and composited by period for each heifer. Feed offered, refusals, and fecal samples were analyzed for DM concentration as described below before calculating DMI and aTTD. Apparent dry matter digestibility (%) and apparent nutrient digestibility (%) were calculated as described by Merchen (1988).

## Digestibility Study 3

A third digestibility study was conducted at the University of Saskatchewan Livestock Research Building (LRB; Saskatoon, SK, Canada) as described by Delver (2023).

**Table 3.** Chemical composition and particle size distribution of diets fed during three digestibility studies (mean ± SD).

| | Study 1[1] | | | | Study 2[2] | | | | Study 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EA | LA | ET | LT | EAET | EALT | LAET | LALT | High | High-mod | Mod | Low |
| Composition[3,4], % DM | | | | | | | | | | | | |
| DM, % | 93.6 ± 1.80 | 93.5 ± 2.10 | 93.8 ± 2.22 | 94.2 ± 1.98 | 91.8 ± 1.67 | 91.7 ± 1.66 | 91.6 ± 1.77 | 91.5 ± 1.75 | 38.7 ± 0.40 | 59.8 ± 0.58 | 71.6 ± 0.60 | 79.4 ± 0.36 |
| OM | 89.5 ± 0.32 | 89.8 ± 0.88 | 89.5 ± 1.02 | 92.8 ± 2.83 | 89.5 ± 0.75 | 92.0 ± 0.64 | 89.5 ± 0.68 | 92.3 ± 1.48 | 93.6 ± 0.06 | 93.4 ± 0.45 | 93.3 ± 0.69 | 93.3 ± 0.72 |
| CP | 18.0 ± 0.34 | 16.0 ± 0.37 | 14.7 ± 0.63 | 10.3 ± 2.98 | 16.0 ± 0.56 | 13.9 ± 0.43 | 15.6 ± 0.60 | 12.5 ± 1.53 | 11.5 ± 0.56 | 11.2 ± 0.00 | 10.8 ± 0.85 | 10.8 ± 0.55 |
| aNDFom | 40.5 ± 0.78 | 53.1 ± 0.97 | 56.8 ± 3.82 | 63.1 ± 3.73 | 51.9 ± 1.54 | 54.2 ± 0.84 | 55.1 ± 3.20 | 59.5 ± 2.6 | 40.7 ± 0.92 | 55.7 ± 1.10 | 61.3 ± 3.48 | 62.9 ± 1.28 |
| ADF | 34.1 ± 0.34 | 45.2 ± 1.62 | 38.3 ± 2.18 | 41.5 ± 1.21 | 37.3 ± 0.92 | 38.8 ± 1.20 | 40.2 ± 1.58 | 41.8 ± 0.96 | 23.1 ± 0.49 | 35.5 ± 0.55 | 39.3 ± 1.63 | 42.6 ± 1.95 |
| ADL | 6.69 ± 0.11 | 9.26 ± 0.23 | 5.96 ± 0.12 | 6.75 ± 0.77 | 5.94 ± 0.28 | 6.48 ± 0.35 | 6.88 ± 0.48 | 7.08 ± 0.30 | 3.4 ± 0.13 | 4.9 ± 0.36 | 5.7 ± 0.26 | 6.0 ± 0.04 |
| uNDF | 20.7 ± 0.76 | 29.1 ± 1.00 | 17.9 ± 1.16 | 23.0 ± 1.76 | 18.8 ± 1.47 | 21.3 ± 1.23 | 21.6 ± 2.12 | 24.1 ± 1.03 | 10.7 ± 1.01 | 18.0 ± 1.58 | 20.0 ± 1.70 | 21.0 ± 0.17 |
| Ca | 1.36 ± 0.050 | 1.54 ± 0.135 | 0.62 ± 0.039 | 0.60 ± 0.269 | 0.85 ± 0.088 | 0.86 ± 0.057 | 0.86 ± 0.088 | 0.78 ± 0.147 | 0.27 ± 0.01 | 0.57 ± 0.02 | 0.57 ± 0.05 | 0.49 ± 0.04 |
| P | 0.25 ± 0.013 | 0.23 ± 0.006 | 0.30 ± 0.017 | 0.21 ± 0.043 | 0.29 ± 0.008 | 0.24 ± 0.010 | 0.28 ± 0.005 | 0.23 ± 0.026 | 0.28 ± 0.00 | 0.17 ± 0.005 | 0.14 ± 0.005 | 0.12 ± 0.00 |
| Particle size, % | | | | | | | | | | | | |
| >19.0 mm | 54.8 ± 2.49 | 57.8 ± 3.01 | 67.8 ± 7.18 | 73.6 ± 7.72 | 63.8 ± 4.39 | 65.3 ± 7.15 | 64.7 ± 3.51 | 66.2 ± 6.27 | 22.1 ± 1.49 | 24.9 ± 1.75 | 26.6 ± 1.75 | 27.2 ± 1.24 |
| 19.0 to 8.0 mm | 20.9 ± 2.91 | 22.3 ± 0.48 | 18.3 ± 3.53 | 14.9 ± 2.75 | 18.8 ± 2.52 | 17.7 ± 3.42 | 18.0 ± 2.65 | 16.9 ± 3.55 | 61.8 ± 0.54 | 46.0 ± 0.85 | 40.0 ± 0.29 | 38.4 ± 0.71 |
| 8.0 to 1.18 mm | 10.3 ± 0.56 | 8.7 ± 1.07 | 7.1 ± 2.10 | 5.7 ± 1.81 | 8.5 ± 1.60 | 8.3 ± 2.34 | 8.2 ± 0.75 | 8.0 ± 1.49 | 10.4 ± 0.61 | 13.3 ± 0.15 | 16.0 ± 0.51 | 16.1 ± 0.07 |
| <1.18 mm | 14.0 ± 2.34 | 11.2 ± 2.12 | 6.7 ± 3.12 | 5.8 ± 3.84 | 9.0 ± 0.84 | 8.7 ± 1.80 | 9.2 ± 2.64 | 8.9 ± 3.60 | 5.7 ± 0.35 | 15.7 ± 0.75 | 17.5 ± 0.96 | 18.3 ± 1.17 |

[1]EA, early alfalfa; LA, late alfalfa; ET, early timothy; LT, late timothy; EAET, early alfalfa × early timothy.
[2]EALT, early alfalfa × late timothy; LAET, late alfalfa × early timothy; LALT, late alfalfa × late timothy.
[3]% of DM unless otherwise stated, [$n$ = 4 (Study 1 and 2); $n$ = 2 (Study 3)].
[4]DM = dry matter, OM = organic matter, CP = crude protein, aNDFom = amylase-treated ash-corrected neutral detergent fiber, ADF = acid detergent fiber, ADL = acid-detergent lignin, uNDF = undigested amylase-treated ash-corrected NDF after 240 h of in vitro incubation, Ca = calcium, P = phosphorus.

*Experimental design* The experiment was designed as a randomized complete block with 18 commercial black Angus cows (571 ± 16 kg initial BW, 656 ± 51 kg end BW) randomly assigned to 2 blocks in 4 consecutive 26 d periods. Cows were housed in individual pens (3 × 3 m) and adapted to each diet for the first 14 d of each period, DMI was measured from day 15 to 21, and fecal samples were collected from day 17 to 21 to estimate aTTD. For DMI measurements, individual feed ingredient samples were collected daily and pooled on an equal weight basis (as is) during the collection period. Refusal samples from each cow were composited on a proportional basis (as is). During fecal collection, cows were tethered to allow for diversion of urine through bladder catheters and to enable scraping of pens and collection of feces every 6 h. The composited feed, refusal, and fecal samples were then dried in a forced-air oven at 55°C until there was no further reduction in sample weight. The amount of feed offered, refused, and fecal output were corrected for the measured DM concentration. The remainder of the experiment was consistent with Study 1 and 2 with the exception that cows were fed once daily at 0800 h, targeting a minimum of 5% refusals, and treatment diets consisted of blends of barley silage, grass hay, wheat straw, and urea formulated to differ in quality as indicated by the concentration of aNDFom (Table 3). Diets were fed in sequential order with diet quality progressively decreasing in each 26 d period.

## Grazing Study

A grazing study was conducted over 2 consecutive summers at the University of Saskatchewan LFCE Cow-Calf Research and Teaching Unit (FCCRTU) near Clavet, Saskatchewan, Canada. Detailed pasture establishment and management, and grazing adaptation is described by Wasden et al. (unpublished) and as described below.

*Experimental design* A 66-hectare (165 acre) field in the dark brown soil zone was divided into twelve paddocks, consisting of four treatments with three replicate paddocks each in a completely randomized block design. Two perennial treatments were established in 2018 comprised of: 1) AC Success hybrid bromegrass (*Bromus riparius* Rehm. × *Bromus inermis* Leyss.) × PS30006 alfalfa (*Medicago sativa* L.) with the botanical composition determined to be 54.8% and 43.8% respectively, and 1.4% other (species not seeded), as determined by the Daubenmire frame method (Daubenmire, 1959); and 2) AC Armada meadow bromegrass (*Bromus riparius* Rehm.) × AAC Mountainview sainfoin (*Onobrychis viciifolia* Scop.) with a botanical composition of 87.3%, 12.4%, respectively and 0.4% other. To control weed growth, the perennial treatment plots were treated with 0.2 L ha$^{-1}$ of glyphosate herbicide approximately mid-April. Plots were under seeded mid-May to AC Rosser barley (*hordeum vulgare*) at a rate of 54 kg ha$^{-1}$ and a seeding depth of 3.8 cm with an application of nitrogen fertilizer at a rate of 56 kg ha$^{-1}$ mid-June. The day after fertilizer application, the perennial seed was planted at a depth of 1.3 cm at a rate recommend for the dark brown soil zone. In two consecutive years (2020 and 2021), two annual treatments were seeded including: 1) AC Hazlet fall rye (*Secale cereale* L.) × Frosty berseem clover (*Trifolium alexandrinum* L.) with the botanical composition (Daubenmire, 1959) of 83.5%, 2.4%, respectively, and 14.1% other (species not seed); and 2) CDC Austenson barley (*Hordeum vulgare* L.) × 4010 pea (*Pisum sativum* L.) × Winfred forage brassica (*Brassica oleracea* L.× *Brassica rapa* L.) × Gorilla forage brassica (*Brassica napus* L.) with the botanical composition determined to be 68.2%, 13.6%, and 11.5% (combined brassica), respectively, and 6.7% other. Weed growth was controlled in the first study year by two pre-seed applications of glyphosate herbicide (0.27 L ha$^{-1}$ early May, and 0.20 L ha$^{-1}$ late May), and in the second year with a mid-May application of 0.27 L ha$^{-1}$. Seed was planted at a depth of 1.3 cm and at rates recommended for the dark brown soil zone. Annual treatments were also fertilized yearly with 13-33-0-15 of N, P, K, and S at a rate of 112 kg ha$^{-1}$.

Pastures were grazed by yearling *Bos taurus* British × Continental crossbred steers (initial BW = 364 ± 14 kg) from July to September over two consecutive years. Steers were adapted pre-trial for a minimum of 21 d on a grass-legume mixture of Kirk crested wheatgrass, AC Success hybrid bromegrass, 3006 alfalfa, Armada meadow bromegrass, and mixed common sainfoin. When forage growth reached approximately 14 cm tall or the 5-6 leaf stage, steers were moved to their respective trial paddock. Continuous grazing management was used, with steers removed once the forage stand height reached 5 cm. Ad libitum water, a 2:1 pre-mix mineral (NLM 2:1 Forage Fortifier Beef Premix NS, New Life Mills, Saskatoon, SK, Canada), and blue cobalt salt blocks were provided. Forage DM yield and nutritive value were obtained by monthly quadrat clipping ($n$ = 40/paddock, 0.25 m$^2$) to a height of 5 cm representative of animal grazing height. Fecal grab samples ($n$ = 13 steers/paddock) were collected between 0800 h and 1000 h and composited by paddock ($n$ = 12) within a day of clipping. Ten sub-samples from the clippings, and the composited fecal samples were then stored at –17 °C until further processing.

## Chemical Analysis

Feed, refusal, and fecal samples from all studies were oven dried at 55 °C for 72 h, ground through a 1.0-mm screen using a Christy & Norris laboratory mill (Christy and Norris, Christy Turner, Ltd., Chelmsford, UK), and retained for chemical analysis. Samples were analyzed for analytical DM (Association of Official Analytical Chemists (AOAC, 1990), method 930.15), OM (AOAC method 942.05), N (AOAC method 990.03), aNDFom (Mertens, 2002), ADF (AOAC method 973.18), ADL (AOAC method 973.18), calcium (Ca) and P. Calcium and P were measured by inductively coupled plasma spectroscopy (ICP). Finally, uNDF was determined after 240 h of rumen in vitro incubation as described by Raffrenato et al. (2018).

## Spectra Collection and Calibration Development

Dried and ground fecal samples (~50 g) were packed into quartz ring cups and scanned in duplicate (two repacks; where the second scan was completely different from the first) using a FOSS DS2500 (FOSS Analytical, Eden Prairie, MN, USA) scanning monochromator. Duplicate NIRS absorption data were collected every 2.0 nm from 400 to 2,498 nm (FOSS), recorded as log 1/$R$, and averaged. Reference data for constituents of interest were matched to the corresponding spectra, which were then divided into two spectral libraries. The first library was comprised of fecal nutrient concentrations and included spectra from all four studies ($n$ = 186). The second library consisted only of samples from the digestibility studies ($n$ = 134) to

predict intake and nutrient digestibility. Spectra were mathematically pre-treated to correct for particle size (standard normal variate (SNV) scatter correction) and baseline offset (detrend (DT) function) (Barnes et al., 1989). The algorithms CENTER and SELECT in WinISI 4.1 software (Infrasoft International, Port Matilda, PA, USA) were used to establish population boundaries by scoring the spectra using a Mahalanobis distance (distance between a sample and the center of the group) of 3.0 (Shenk and Westerhaus, 1991).

Modified partial least-squares (MPLS) regression in WinISI 4.1 software was used to develop the equations, during which two mathematical treatments were tested: 1, 4, 4, 1 and 2, 6, 5, 1; with the first digit representing the derivative, the second digit the gap over which the derivative was calculated, the third was the number of data points used in the running average for smoothing of derivative spectra, and the fourth was the number of data points over which the second smoothing was applied (ISI, 1999).

In the absence of external datasets, internal cross-validation was used to validate all calibrations in which one-fifth of the calibration samples were randomly selected and used to validate calibrations calculated in the remaining four-fifths of the sample library (Landau et al., 2016; Jancewicz et al., 2017a). The optimal math treatment for each constituent of interest was determined by selecting the coefficient of determination of cross-validation ($R^2_{cv}$) closest to 1.0, and the smallest possible standard error of cross-validation (SECV, calculated as the average SEC of every subset). Jancewicz et al. (2017b) applied Gaussian distribution theory to SECV to estimate prediction error in either 68% or 95% of the samples and the same criteria was applied to the present study. Additionally, for fecal nutrient content a $R^2_{cv}$ of > 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 (Jancewicz et al., 2017b). Digestibility and intake are not chemical characteristics, but rather properties of the forage requiring either in vivo or in vitro measurements (Stuth et al., 2003) which can result in increased error. For this reason the criteria for intake and digestibility were more lenient with an $R^2_{cv}$ > 0.70 considered as good (Landau et al., 2016; Jancewicz et al., 2017b).

Principal component analysis (PCA) was used to visualize the differences in spectral population between each study, similar to Jancewicz et al. (2017b). Spectra were imported into Unscrambler X Version 10.3 (CAMO Software 2010) and trimmed to 1,108 to 2,498 nm, to exclude wavelengths in the visible region. Spectra was then pretreated (DT and SNV) and a derivative treatment applied (1,5,5,1). Four samples from the annual treatment (fall rye × berseem clover) of the first grazing year were identified as extreme outliers and removed from the PCA. A Hotelling's $T^2$ ellipse was used to identify samples that are similar within a 95% confidence limit. Finally, a principal component score scatter plot was graphed along the first two principal components (x-axis = PC1, y-axis = PC2).

## Results

### Calibration for Fecal Chemical Composition

Calibration equations for fecal composition were developed using the first spectral library ($n$ = 186) including samples from all four studies. The PCA plot indicates that the data set belonged primarily within the same population; however, diversity was also evident based on distribution along the first and second components (Fig 1.). Outliers identified in the PCA were related to annual treatments (fall rye × berseem clover) of the second year of the grazing study (bottom right of Fig. 1). Additionally, summary statistics for calibration and cross-validation of fecal nutrient NIRS equations are presented in Table 4. The first derivative math treatment (1,4,4,1) produced calibration equations with the highest $R^2_{cal}$ and lowest SEC for the determination of fecal OM, N, ADL, uNDF and P, while the first derivative treatment (2,6,5,1) yielded the best results for aNDFom, ADF and Ca. The calibrations resulted in excellent agreement between reference

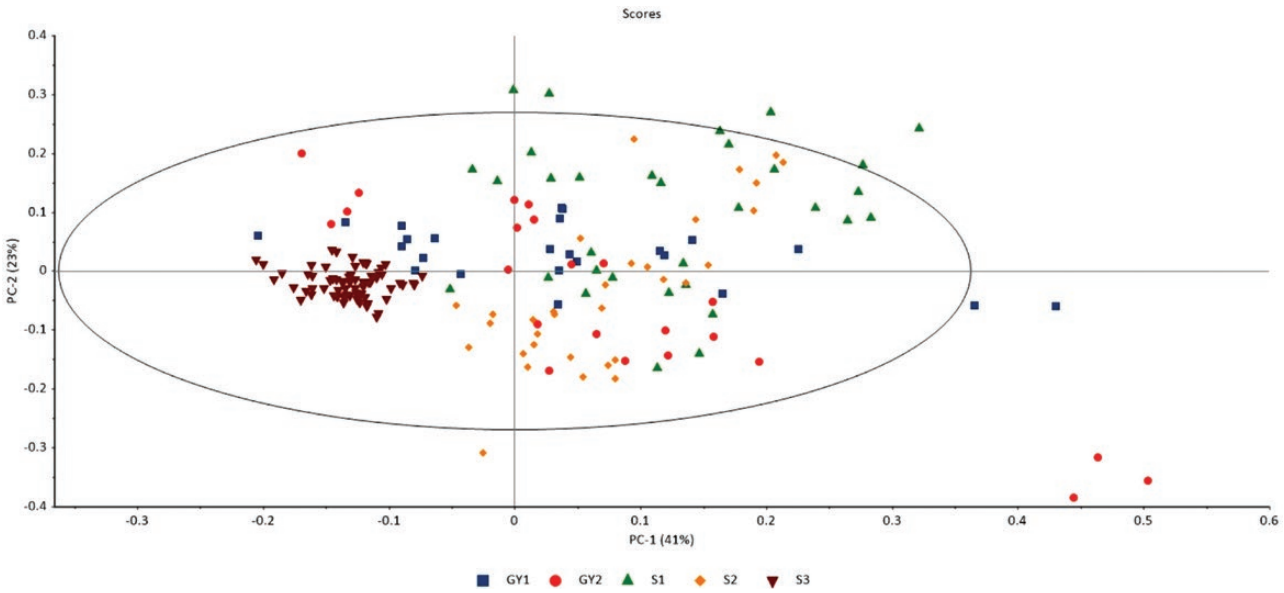


**Figure 1.** Principle component plot of the full library of spectra including three digestibility studies (S1, S2, and S3) and two-years of grazing samples (GY1 and GY2).

**Table 4.** Near infrared spectroscopy calibration statistics determined by modified partial least-squares (MPLS) regression for fecal composition using the first spectral library ($n$ = 186).

| | | Range | Calibration statistics[2] | | | | Cross validation statistics[3] | |
|---|---|---|---|---|---|---|---|---|
| | $n$ | [min-max(mean)] | Terms | MthTrt | $R^2_{cal}$ | SEC | $R^2_{cv}$ | SECV |
| Composition[1], % DM | | | | | | | | |
| OM | 158 | 81.3-91.1 (87.2) | 11 | 1,4,4,1 | 0.98 | 1.40 | 0.96 | 1.88 |
| N | 164 | 1.55-2.48 (2.00) | 8 | 1,4,4,1 | 0.95 | 0.06 | 0.94 | 0.07 |
| aNDFom | 164 | 48.8-66.8 (57.7) | 10 | 2,6,5,1 | 0.93 | 1.31 | 0.88 | 1.70 |
| ADF | 161 | 34.0-50.7 (44.2) | 6 | 2,6,5,1 | 0.95 | 0.98 | 0.94 | 1.10 |
| ADL | 160 | 8.20-20.2 (15.2) | 10 | 1,4,4,1 | 0.96 | 0.53 | 0.95 | 0.61 |
| uNDF | 164 | 29.8-55.4 (43.1) | 9 | 1,4,4,1 | 0.92 | 1.70 | 0.89 | 2.00 |
| Ca | 161 | 0.70-4.54 (2.12) | 11 | 2,6,5,1 | 0.98 | 0.14 | 0.97 | 0.18 |
| P | 164 | 0.31-0.97 (0.56) | 8 | 1,4,4,1 | 0.89 | 0.05 | 0.86 | 0.06 |

[1]OM = organic matter, N = nitrogen, aNDFom = amylase-treated ash-corrected neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin, uNDF = amylase-treated ash-corrected neutral detergent fiber after 240hr, Ca = calcium, P = phosphorus.
[2]Terms = number of scores used in the calibration, MthTrt = spectral math treatment providing the best calibration statistics, $R^2_{cal}$ = coefficient of determination of calibration, SEC = standard error of calibration.
[3]$R^2_{cv}$ = coefficient of determination of cross-validation, SECV = standard error of cross-validation.

and predicted values for OM ($R^2_{cv}$ = 0.96), N ($R^2_{cv}$ = 0.94), ADF ($R^2_{cv}$ = 0.94), ADL ($R^2_{cv}$ = 0.95), and Ca ($R^2_{cv}$ = 0.97), with good agreement for aNDFom, uNDF and P with $R^2_{cv}$ of 0.88, 0.89, and 0.86, respectively. Equations resulted in error terms (SECV) of 1.88% for OM, 0.07% for N, 1.70% for aNDFom, 1.10% for ADF, 0.61% for ADL, 2.00% for uNDF, 0.18% for Ca, and 0.06% for P.

### Calibrations for Intake and Nutrient Digestibility

Calibration equations for intake and nutrient digestibility were developed using the second spectral library ($n$ = 134) which included intake and digestibility data from the three digestibility studies. A smaller sample set resulted in less spectral diversity, as indicated in Figure 1. The second derivative math treatment (2,6,5,1) produced calibrations with the highest $R^2_{cal}$ and lowest SEC for the prediction of DM, OM, N, aNDFom and uNDF intake (kg·d$^{-1}$, % BW, g·kg$^{-1}$ BW$^{0.75}$) and ADL (kg·d$^{-1}$) while the first derivative treatment (1,4,4,1) yielded the best results for ADL (% BW, g·kg$^{-1}$ BW$^{0.75}$) and the digestibility of DM, OM, aNDFom and N (Table 5).

The cross validation statistics for calibration equations resulted in excellent agreement ($R^2_{cv} \geq 0.81$) between reference and predicted values for intake of DM (kg·d$^{-1}$), OM (kg·d$^{-1}$), N (% BW; g·kg$^{-1}$ BW$^{0.75}$), aNDFom (kg·d$^{-1}$), ADL (% BW; g·kg$^{-1}$ BW$^{0.75}$) and uNDF (% BW), with good agreement ($0.74 \leq R^2_{cv} \leq 0.77$) for intake of DM (% BW), OM (% BW), N (kg·d$^{-1}$) and uNDF (g·kg$^{-1}$ BW$^{0.75}$). Calibrations of moderate agreement ($0.60 \leq R^2_{cv} \leq 0.68$) were developed for the intake of DM (g·kg$^{-1}$ BW$^{0.75}$), OM (g·kg$^{-1}$ BW$^{0.75}$), aNDFom (% BW; g·kg$^{-1}$ BW$^{0.75}$) and uNDF (kg·d$^{-1}$), and poor for ADL (kg·d$^{-1}$, $R^2_{cv}$ = 0.59). Accuracy for constituents resulted in SECV values of 1.12, 1.10, 0.02, 0.69, 0.06 and 0.24 for DM, OM, N, aNDFom, ADL, and uNDF (kg·d$^{-1}$), respectively. Accuracy for calibrations of DM, OM, N, aNDFom, ADL and uNDF (% BW) resulted in SECV values of 0.16, 0.15, 0.00, 0.11, 0.01, and 0.04 respectively. Expressing nutrient intake based on g·kg$^{-1}$ BW$^{0.75}$ did not improve $R^2_{cv}$ values.

The calibrations for aTTD of DM, OM, aNDFom and N yielded moderate to good linearity between reference and predicted values, with $R^2_{cv}$ of 0.72, 0.65, 0.74, and 0.69, respectively and high accuracy (SECV ≤ 2.82; Table 5).

## Discussion

### Calibrations for Fecal Chemical Composition

With an increasing emphasis on grazing productivity and sustainability comes an equally increasing demand for information regarding grazing forage quality. Determining the nutritive value of forages is difficult particularly under grazing situations where environmental conditions can result in rapid changes in forage composition. Chemical constituents can also vary considerably between stems, leaves and flowers, a contributing factor to selective grazing. Near infrared spectroscopy provides an alternative to wet chemistry when determining the composition of feces from cattle fed a wide range of diets (Coleman and Windham, 1989; Lyons and Stuth, 1992; Landau et al., 2004; Dixon and Coates, 2009; Jancewicz et al., 2017b; Johnson et al., 2017; Simoni et al., 2021). The development of robust predictive equations requires samples from the full range of diets that may be consumed. The present study used fecal samples from cattle consuming forage of various types, quality, and under confined tie-stall controlled environment and grazing systems to increase the range of samples available to populate calibrations that estimate fecal composition.

Prediction equations reported in this study are comparable or stronger than previously reported statistics for dried and ground cattle feces. In a recent feedlot study, Jancewicz et al. (2017b) reported strong cross validation statistics for fecal composition with $R^2_{cv}$ values of 0.90 and 0.97 and SECV values of 1.02 and 0.10 for OM and N, respectively. These values compare closely to those reported in the present study with $R^2_{cv}$ of 0.96 and 0.93 with SECV values of 1.73 and 0.07 for OM and N. Brogna et al. (2018) also recently reported strong cross validation statistics for fecal

**Table 5.** Near infrared spectroscopy calibration statistics determined by modified partial least-squares (MPLS) regression for intake of nutrients and nutrient digestibility using the second spectral library ($n$ = 134).

| | | | Calibration statistics[2] | | | | Cross validation statistics[3] | |
|---|---|---|---|---|---|---|---|---|
| | *n* | Range (mean) | Terms | MthTrt | $R^2_{cal}$ | SEC | $R^2_{cv}$ | SEV |
| Intake, kg·d$^{-1}$ | | | | | | | | |
| DM | 131 | 3.91-16.9 (10.9) | 7 | 2,6,5,1 | 0.89 | 0.92 | 0.84 | 1.12 |
| OM | 131 | 3.47-15.8 (10.0) | 7 | 2,6,5,1 | 0.89 | 0.89 | 0.83 | 1.10 |
| N | 131 | 0.08-0.34 (0.22) | 7 | 2,6,5,1 | 0.83 | 0.02 | 0.74 | 0.02 |
| aNDFom | 134 | 2.27-8.89 (5.77) | 8 | 2,6,5,1 | 0.89 | 0.54 | 0.81 | 0.69 |
| ADL | 132 | 0.30-0.79 (0.59) | 7 | 2,6,5,1 | 0.71 | 0.05 | 0.59 | 0.06 |
| uNDF | 132 | 0.91-2.84 (1.96) | 6 | 2,6,5,1 | 0.77 | 0.21 | 0.68 | 0.24 |
| % BW | | | | | | | | |
| DM | 129 | 1.36-2.97 (2.14) | 9 | 2,6,5,1 | 0.88 | 0.12 | 0.77 | 0.16 |
| OM | 131 | 1.25-2.63 (1.96) | 10 | 2,6,5,1 | 0.88 | 0.11 | 0.75 | 0.15 |
| N | 129 | 0.03-0.09 (0.05) | 8 | 2,6,5,1 | 0.94 | 0.00 | 0.91 | 0.00 |
| aNDFom | 131 | 0.70-1.67 (1.13) | 9 | 2,6,5,1 | 0.82 | 0.08 | 0.67 | 0.11 |
| ADL | 128 | 0.06-0.22 (0.12) | 6 | 1,4,4,1 | 0.92 | 0.01 | 0.91 | 0.01 |
| uNDF | 131 | 0.20-0.67 (0.40) | 10 | 2,6,5,1 | 0.94 | 0.03 | 0.85 | 0.04 |
| g·kg$^{-1}$ BW$^{0.75}$ | | | | | | | | |
| DM | 131 | 59.2-132.5 (100.9) | 6 | 2,6,5,1 | 0.73 | 7.58 | 0.66 | 8.56 |
| OM | 131 | 52.7-124.0 (92.8) | 7 | 2,6,5,1 | 0.77 | 6.67 | 0.64 | 8.22 |
| N | 130 | 1.20-3.96 (2.14) | 9 | 2,6,5,1 | 0.94 | 0.13 | 0.86 | 0.19 |
| aNDFom | 132 | 31.3-73.6 (53.5) | 6 | 2,6,5,1 | 0.7 | 4.60 | 0.60 | 5.33 |
| ADL | 128 | 3.24-9.32 (5.64) | 8 | 1,4,4,1 | 0.88 | 0.39 | 0.84 | 0.45 |
| uNDF | 128 | 10.6-28.9 (18.6) | 9 | 2,6,5,1 | 0.91 | 1.14 | 0.76 | 1.86 |
| Digestibility, % | | | | | | | | |
| DM | 130 | 52.1-74.3 (63.1) | 9 | 1,4,4,1 | 0.81 | 1.82 | 0.72 | 2.20 |
| OM | 130 | 55.2-75.7 (65.0) | 7 | 1,4,4,1 | 0.75 | 1.95 | 0.65 | 2.29 |
| aNDFom | 131 | 45.0-71.3 (59.8) | 9 | 1,4,4,1 | 0.81 | 2.17 | 0.74 | 2.51 |
| N | 128 | 38.6-77.7 (64.5) | 4 | 1,4,4,1 | 0.72 | 2.70 | 0.69 | 2.82 |

[1]DM = dry matter, OM = organic matter, N = nitrogen, aNDFom = amylase-treated ash-corrected neutral detergent fiber, ADL = acid detergent lignin, uNDF = amylase-treated ash-corrected neutral detergent fiber after 240hr of in vitro incubation.
[2]Terms = number of scores used in the calibration, MthTrt = spectral math treatment providing the best calibration statistics, $R^2_{cal}$ = coefficient of determination of calibration, SEC = standard error of calibration.
[3]$R^2_{cv}$ = coefficient of determination of cross-validation, SECV = standard error of cross-validation.

composition from dairy diets with $R^2_{cv}$ of 0.87 and 0.88, and SECV values of 1.89 and 2.07 for aNDFom and ADF, respectively. By comparison, we report $R^2_{cv}$ values of 0.95 and 0.95, with SECV values of 1.65 and 1.20 for the same constituents (aNDFom and ADF). As mentioned, the inclusion of fecal samples collected from cattle fed a wide variety of harvested and fresh grazed forage sources provided spectral diversity and increased robustness of the calibration equation. However, the stronger calibration statistics for fecal composition may be related to decreased ranges in the current study for OM, N, aNDFom, and ADF of 81.3% to 91.1%, 1.55% to 2.48%, 48.8% to 66.8%, and 34.0% to 50.7%, respectively. In contrast, Jancewicz et al. (2017b) included a range for OM and N of 57.4% to 91.0%, and 0.8% to 4.2%, with Brogna et al. (2018) including ranges of 43.9% to 82.0% and 28.7% to 85.4% for aNDFom and ADF, respectively. This may be further confounded by the larger sample sizes reported by Jancewicz et al (2017b) and Brogna et al. (2018) of 248 and 301 samples, respectively, in comparison to the 186 samples included in the present calibration.

Furthermore, error terms for the fecal composition equations were comparable or lower than previously reported and notably improved for the fiber predictions. Brogna et al. (2018) indicated that the main source of inaccuracy in the calibration process is sampling and internal laboratory error. We compiled data from several studies running simultaneously at the same research facility, which contributed to increased consistency in sample collection and processing. Furthermore, the chemical analysis was performed by the same laboratory, reducing laboratory bias, a challenge often faced during the NIRS calibration process. Combined, these steps were expected to reduce analysis error across samples which is reflected in the low error terms of the prediction equations. However, similar control methods were employed by Righi et al. (2017) and Simoni et al. (2021) who were unsuccessful in creating fecal composition calibration equations of similar quality to those reported here.

The SEC error terms for OM reported by Jancewicz et al. (2017b) and Purnomoadi et al. (1996) are lower than those reported in the current calibration with 0.79, 1.29, and 1.48, respectively. This could be related to the forage inclusion

level in the diet of each study as the fecal calibrations reported by Jancewicz et al. (2017b) were based on beef cattle backgrounding and finishing feedlot diets. Purnomoadi et al. (1996) calibrations were derived from dairy TMR diets, and the calibration reported here were from diets with > 95% of forage on a DM basis. The ash content of forage, harvested or ingested, can be substantial depending on the time it was harvested or grazed and its degree of contamination with soil. Several samples from the current grazing study were identified as spectral outliers due to excessive soil contamination identified by the high ash content as a result of overgrazing (Fig. 1 and data not shown). Fecal ash calibration statistics reported by Landau et al. (2016) of $R^2_{cv}$ = 0.94 (SECV = 1.30) from grazing beef animals are closer to the calibration statistics for OM reported here, supporting the theory that samples of higher ash or lower organic matter will result in poorer calibrations.

Few prediction equations for fecal mineral content have been developed, likely because minerals that are not part of an organic bond do not absorb energy in the near infrared region and are found in low concentrations. Recently, Ikoyi and Younge (2022) reported $R^2_{cv}$ statistics in equine feces for Ca and P of 0.26 and 0.71, and SECV of 2.59 and 0.43. The present study $R^2_{cv}$ statistics for Ca and P of 0.97 and 0.85 with SECV of 0.18 and 0.06 are unusually strong, although Showers et al. (2006) reported a fecal P $R^2_{cv}$ of 0.81 and SECV of 0.04 in white-tailed deer (*Odocoileu virginianus*). These studies included diets with supplementary mineral sources, thus the mineral source was not entirely organic, supporting the potential of NIRS as an indicator of mineral content. With increasing focus on fertilizer use and environmental sustainability, future work in this area is warranted.

Internal markers such as acid insoluble ash, ADL, and uNDF can be used as predictors of diet quality and intake of grazing animals (Decruyenaere et al., 2009; Ovani et al., 2022). Calibration statistics developed in the current study for ADL and uNDF resulted in $R^2_{cv}$ of 0.96, and 0.93 and SECV values of 0.63 and 1.91. Brogna et al. (2018) also recently reported strong cross validation statistics for fecal composition from dairy diets with $R^2_{cv}$ of 0.85 and 0.86 and SECV values of 2.05, and 2.24 for ADL and uNDF, respectively. Our results support the results of Brogna et al. (2018) which indicated that in addition to ADL, uNDF could be used as an internal marker to estimate intake and digestibility. Buonaiuto et al. (2021) also recently developed a NIRS calibration equation to predict uNDF in the TMR fed to dairy cows that generated similar statistics ($R^2_{cv}$ = 0.74, SECV = 1.98) to those in the present study. In contrast, the uNDF fecal calibration statistics of Righi et al. (2017) and Simoni et al. (2021) were poorer, with $R^2_{cv}$ of 0.66 and 0.45, and SECV of 3.27 and 3.40, respectively. However, the range of fecal uNDF reported in those studies (26.7% to 54.2% and 21.0% to 52.6%, respectively) includes lower concentrations than those reported in the current study and by Brogna et al. (2018) (29.8% to 55.4%, and 27.4% to 59.4%, respectively). This suggests that higher concentrations of fecal uNDF will result in more robust calibration statistics. Jancewicz et al. (2017b) reported a similar reduction in ADL validation statistics ($R^2_{val}$ = 0.70) in comparison to the $R^2_{cv}$ = 0.92 and suggested that a low range in fecal ADL concentration (0.7% to 13%) was responsible. This reduction in prediction ability could also reflect the small sample size ($n$ = 55) as compared to the current study ($n$ = 186).

## Calibrations for Nutrient Intake

Determining nutrient intake of grazing cattle is one of the greatest limitations to optimizing grazing pasture systems for long term efficiency and environmental sustainability. Most methods for estimating intake in grazing ruminants are difficult to implement, time consuming, and alter grazing behaviour. Norris et al. (1976) pioneered the prediction of forage DMI from direct NIRS spectra of feeds with an error of 7.8 g·kg$^{-1}$ BW$^{0.75}$, a value promising enough to warrant further investigation. Decruyenaere et al (2004) reported a SECV of 6.78 g·kg$^{-1}$ BW$^{0.75}$, smaller than the SECV of 8.6 g·kg$^{-1}$ BW$^{0.75}$ reported in the current study. In contrast, recent studies by Landau et al. (2016) and Johnson et al. (2017) reported poorer SECV terms of 10.2 and 11.1 g·kg$^{-1}$ BW$^{0.75}$, respectively. In agreement with our findings, Landau et al. (2016) reported calibration statistics for nutrient intake were poorer than those for fecal composition.

We report the strongest prediction statistics for DM, OM, and aNDFom intake on a kg·d$^{-1}$ animal$^{-1}$ basis with $R^2_{cv}$'s of 0.84, 0.83, and 0.81 and SECV 1.12, 1.10, and 0.69, respectively. Landau et al. (2016) reported $R^2_{cv}$ calibration statistics for DM and aNDFom kg·d$^{-1}$ of 0.75 and 0.65 and SECV error terms of 1.2 and 0.63, respectively. To our knowledge, ours is the first report of a fecal NIRS calibration for the intake of OM on a kg·d$^{-1}$ basis. Collectively, these results indicate that expression of intake of these parameters on a kg·d$^{-1}$ is better predictor than % BW or g kg$^{-1}$ BW$^{0.75}$. As noted by Landau et al. (2016), intake is a function of physiological status and gastro-intestinal size, with the latter closely related to BW. Therefore, we would intuitively expect intake and subsequently prediction equations expressed as a % BW or BW$^{0.75}$ to be more accurate. Landau et al, (2016) suggested this unexpected result was due to the variation in dietary and physiological factors of voluntary intake; however, difficulties in the consistent and accurate measurement of BW, as a result of variation in gut fill, could be a confounding factor. Additionally, a limitation of NIRS is that all errors are amplified at the chemometric step and intakes based on BW may already contain inherent error, contributing to poorer calibration statistics. Furthermore, prediction equations for intake, regardless of their unit of measurement, must be linked to one or more chemical constituents containing organic bonds that respond to NIRS enabling them to act as an internal marker within the feces. For these reasons, calibration equations based on kg·d$^{-1}$ may result in stronger statistics as the impact of BW measurement variability is reduced and the impact of fecal composition, which tends to be easier to measure, is increased. This theory may be supported by the stronger calibration statistics reported in the current study and related to increased ranges in the current study for DM and aNDFom intake kg·d$^{-1}$ of 3.9 to 16.9, and 2.3 to 8.9, respectively. In comparison, intakes of Landau et al. (2016) ranged from 5.8 to 10.8, and 2.9 to 5.1 for the intake of DM and aNDFom kg·d$^{-1}$, respectively.

To our knowledge, there are no documented NIRS calibrations for intake of ADL or uNDF. In the present study, the strongest prediction statistics for intake of ADL and uNDF were on a % BW basis with $R^2_{cv}$ of 0.91 and 0.85 and SECV of 0.01 and 0.04. Garnsworthy and Unal (2004) noted that predictions of intake were stronger than those for digestibility and suggested one reason could be that an indigestible component fed at a fixed rate is more readily distinguished from those that are digestible, with these fractions being differentiated by the NIRS spectrum. Due to the study design,

care was taken to select forages of varying quality (e.g., ADL and uNDF) that were fed ad libitum so as to broaden and increase the robustness of calibration equations. Conversely, the range of uNDF intake % BW spanned from 0.20 to 0.67, which is much higher than the maximum uNDF intake of ~0.39% BW reported by van Amburgh et al. (2015) for lactating dairy cattle fed high or low forage diets.

### Calibrations for Nutrient Digestibility

Near infrared spectroscopy has been used extensively to predict forage quality; however, grazing selectively by ruminants diminishes the ability of pasture managers to use this information to predict nutrient intake. More recently, NIRS has successfully predicted digestibility coefficients for DMD, OMD, aNDFom, and N (Boval et al., 2004; Coates and Dixon, 2011; Jancewicz, et al., 2017b; Johnson et al., 2017) in beef cattle fed diets in confinement, suggesting NIRS may be used in a similar manner to predict the quality of grazing forages. However, Purnomoadi et al. (1997) demonstrated that this approach was only viable when feces from dairy cattle consuming the forage or diet of interest were used to develop the calibration equation. Our objective was to use total collection studies using forages of varying quality to build fecal NIRS calibrations capable of accurately predicting the digestibly of forages of varying quality.

The NIRS calibrations for DMD and OMD were found to be stronger when they encompassed the full range of values that were generated across different diet types (Landau et al., 2016). The DMD in the current calibration data ranged from 52.1 to 74.3%, whereas Jancewicz et al. (2017b) ranged from 59.4% to 90.1%, Johnson et al. (2017) from 47.4% to 82.6%, and Landau et al. (2016) from 49.3% to 70.5%. These three studies reported $R^2_{cv}$ of 0.75, 0.82, and 0.89 and SECV of 2.88, 4.31, and 3.1, while the current study produced calibration statistics of $R^2_{cv}$ of 0.72 and an SECV of 2.20. The OMD in the current calibration dataset ranged from 55.2 to 75.7%, from 54.7.4% to 91.0% by Jancewicz et al. (2017b) and from 62% to 70% by Boval et al. (2004), with the later two studies generating $R^2_{cv}$ of 0.77 and 0.69 and SECV of 2.88 and 2.0, respectively. The calibration statistics of the current study are comparable with a $R^2_{cv}$ of 0.65 and an SECV of 2.29. Although our calibration statistics are comparable to those of others, they may indicate a slightly lower precision, but increased accuracy. The reduction in precision may be related to the smaller range in DMD and OMD in the current calibrations, and the improved accuracy related to close control of sampling and laboratory error. We concur with Boval et al. (2004), developing a more robust calibration with an expanded range of DMD and OMD will likely improve the precision by increasing spectral diversity but also increase error as a wider range of diets, animal variability, sample and laboratory error may be encountered.

Since Oba and Allen (1999) identified the positive effect of NDFD on intake and thus on dairy cow production performance, the use of NDFD to assess forage quality has become common. Grazing cattle may select for more digestible portions of the plant in the field resulting in variation in nutrient intake that may not be captured by analysis of forage clippings. The direct prediction of grazing forage NDFD by fecal NIRS would be of high value as it more accurately predicted the quality of the forage being ingested. Complicating the goal is the complexity of accurately quantifying fiber fractions which consist of a complex mixture of chemical components that change during plant growth and with different environment conditions that are not well delineated (Brogna et al., 2018). Reducing sampling and laboratory error, as well as the inclusion of forages of varying aNDFom quality, are critical to developing viable NIRS calibrations. In our study, forage quality of the total collection studies used in the NDFD calibration ranged from 45.0% to 71.3% and resulted in calibration statistics of $R^2_{cv}$ of 0.74 and SECV of 2.51. In contrast, the NDFD calibrations of Jancewicz et al. (2017b) ranged from 26.4% to 76.2%, with $R^2_{cv}$ of 0.33 and SECV 7.9. Jancewicz et al. (2017b) noted a challenge in predicting NDFD directly from feces and suggested that growing beef diets that included by-products may have influenced results. Expanding the range of samples could increase the application of the equations in broader conditions; however, the current calibrations show potential for predicting intake and digestibility of nutrients from the feces of beef cattle fed forage diets.

## Conclusion

In conclusion, by using forages of varying quality and digestibility it was possible to develop moderate to excellent NIRS calibrations for fecal composition, intake, and digestibility from beef cattle fed high forage diets. Accurate and precise predictions for fecal uNDF and ADL support the possibility of internal marker calculated intake and digestibility for grazing beef cattle. Care was taken to include forages ranging in quality and digestibility; however, additional grazing system samples are required to increase the robustness of calibrations. Future steps include the expansion and further validation of the calibrations with spectra from varying forage species and grazing systems.

## Acknowledgments

The financial support provided by the Alberta Beef Producers (Grant FRG.12.19) is gratefully acknowledged. Thanks also to Justin Delver and Megan Wasden for the generous sharing of data. Finally, the authors would also like to thank Nikita Payne, Tyen Patterson, and Phoebe Johnson for their assistance with animal care, sample collection and laboratory analysis.

## Conflict of Interest Statement

The authors disclose that there are no conflicts of interest.

## References

AOAC. 1990. *Official methods of analysis*. 15th ed. Washington, DC: Association of Official Analytical Chemists.

Barnes, R. J., M. S. Dhanoa, and S. J. Lister. 1989. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *App. Spec.* 43(5):772–777. doi:10.1366/0003702894202201

Basarab, J. A., K. A. Beauchemin, V. S. Baron, K. H. Ominski, L. L. Guan, S. P. Miller, and J. J. Crowley. 2013. Reducing GHG emissions through genetic improvement for feed efficiency: effects on economically important traits and enteric methane production. *Animal*. 7(S2):303–315. doi:10.1017/S1751731113000888.

Boval, M., D. B. Coates, P. Lecomte, V. Decruyenaere, and H. Archimède. 2004. Faecal near infrared reflectance spectroscopy (NIRS) to assess chemical composition, in vivo digestibility and intake of

tropical grass by Creole cattle. *Anim. Feed Sci. Technol*. 114:19–29. doi:10.1016/j.anifeedsci.2003.12.009.

Brogna, N., A. Palmonari, G. Canestrari, L. Mammi, A. Dal Prà, and A. Formigoni. 2018. Technical note: near infrared reflectance spectroscopy to predict fecal indigestible neutral detergent fiber for dairy cows. *J. Dairy Sci*. 101:1234–1239. doi:10.3168/jds.2017-13319.

Buonaiuto, G., D. Cavallini, L. M. E. Mammi, F. Ghiaccio, A. Palmonari, A. Formigoni, and G. Visentin. 2021. The accuracy of NIRS in predicting chemical composition and fibre digestibility of hay-based total mixed rations. *Ital. J. Anim. Sci*. 20:1730–1739. doi:10.1080/1828051x.2021.1990804.

Coates, D. B., and R. M. Dixon. 2011. Developing robust faecal near infrared spectroscopy calibrations to predict diet dry matter digestibility in cattle consuming tropical forages. *J. Near Infrared Spectrosc*. 19:507–519. doi:10.1255/jnirs.967.

Coleman, S. W., and W. R. Windham. 1989. Measurements of forage quality. near infrared reflectance spectroscopy (NIRS): analysis of forage quality. 643:83.

Daubenmire, R. 1959. A canopy-coverage method of vegetational analysis. *Northwest Sci*. 33:43–64.

Decruyenaere, V., A. Buldgen, and D. Stilmant. 2009. Factors affecting intake by grazing ruminants and related quantification methods: a review. *Biotechnol. Agron. Soc. Environ*. 13:559–573.

Decruyenaere, V., M. De Visser, D. Stilmant, C. Clement, and G. Sinnaeve. 2004. Faecal near infrared reflectance spectroscopy for ruminant feed intake prediction. *Proc of the 20th Gen. Meet. Europ. Grass. Fed*. 9:1034–1036.

Delver, J. J. 2023. Development of an efficiency ranking system for beef cows and a comparison of the digestive physiology between efficient and inefficient cows. [Master of Science Thesis]. Saskatoon: University of Saskatchewan, Harvest. https://hdl.handle.net/10388/14649.

Dixon, R., and D. Coates. 2009. Review: near infrared spectroscopy of faeces to evaluate the nutrition and physiology of herbivores. *J. Near Infrared Spectrosc*. 17:1–31. doi:10.1255/jnirs.822.

Ellis, W. C. 1978. Determinants of grazed forage intake and digestibility. *J. Dairy Sci.* 61(12):1828–1840. doi:10.3168/JDS.S0022-0302(78)83809-0.

Garnsworthy, P. C., and Y. Unal. 2004. Estimation of dry-matter intake and digestibility in group- fed dairy cows using near infrared reflectance spectroscopy. *Anim. Sci*. 79:327–334. doi:10.1017/s1357729800090184.

Harper, K. J., and D. M. McNeill, 2015. The role iNDF in the regulation of feed intake and the importance of its assessment in subtropical ruminant systems. *Agriculture*. 5(3):778–790. doi:10.3390/AGRICULTURE5030778.

Huhtanen, P., U. Asikainen, M. Arkkila, and S. Jaakkola. 2007. Cell wall digestion and passage kinetics estimated by marker and in situ methods or by rumen evacuations in cattle fed hay 2 or 18 times daily. *Anim. Feed Sci. Technol*. 133:206–227. doi:10.1016/j.anifeedsci.2006.05.004.

Ikoyi, A. Y., and B. A. Younge. 2022. Faecal near-infrared reflectance spectroscopy profiling for the prediction of dietary nutritional characteristics for equines. *Anim. Feed Sci. Technol*. 290:115363. doi:10.1016/j.anifeedsci.2022.115363.

ISI. 1999. WinISI, the complete software solution for routine analysis, robust calibrations and networking (1.02A). Infrasoft International.

Jancewicz, L. J., G. B. Penner, M. L. Swift, C. L. Waldner, K. M. Koenig, K. A. Beauchemin, and T. A. McAllister. 2017a. Predicting fecal nutrient concentrations and digestibilities and growth performance in feedlot cattle by near-infrared spectroscopy. *J. Anim. Sci*. 95:455–474. doi:10.2527/jas.2016.0876.

Jancewicz, L. J., M. L. Swift, G. B. Penner, K. A. Beauchemin, K. M. Koenig, G. E. Chibisa, M. L. He, J. J. McKinnon, W. Z. Yang, and T. A. McAllister. 2017b. Development of near- infrared spectroscopy calibrations to estimate fecal composition and nutrient digestibility in beef cattle. *Can. J. Anim. Sci*. 97:51–64. doi:10.1139/CJAS-2016-0107.

Johnson, J. R., G. E. Carstens, S. D. Prince, K. H. Ominski, K. M. Wittenberg, M. Undi, T. D. A. Forbes, A. N. Hafla, D. R. Tolleson, and J. A. Basarab. 2017. Application of fecal near- infrared reflectance spectroscopy profiling for the prediction of diet nutritional characteristics and voluntary intake in beef cattle. *J. Anim. Sci*. 95:447–454. doi:10.2527/jas.2016.0845.

Kalu, B. A., and G. W. Fick. 1981. Quantifying morphological development of alfalfa for studies of herbage quality 1. *Crop Sci*. 21:267–271. doi:10.2135/cropsci1981.0011183x002100020016x.

Kononoff, P. J., A. J. Heinrichs, and D. R. Buckmaster. 2003. Modification of the Penn State Forage and total mixed ration particle separator and the effects of moisture content on its measurements. *J. Dairy Sci*. 86:1858–1863. doi:10.3168/jds.S0022-0302(03)73773-4.

Landau, S. Y., L. Dvash, M. Roudman, H. Muklada, D. Barkai, Y. Yehuda, and E. D. Ungar. 2016. Faecal near-IR spectroscopy to determine the nutritional value of diets consumed by beef cattle in east Mediterranean rangelands. *Animal*. 10:192–202. doi:10.1017/S175173111500169X.

Landau, S., T. Glasser, L. Dvash, and A. Perevolotsky. 2004. Faecal NIRS to monitor the diet of Mediterranean goats. *S. Afr. J. Anim. Sci*. 34:76–80.

Lippke, H., W. C. Ellis, and B. F. Jacobs. 1986. Recovery of indigestible fiber from feces of sheep and cattle on forage diets. *J. Dairy Sci*. 69:403–412. doi:10.3168/jds.S0022- 0302(86)80418-0.

Lyons, R. K., and J. W. Stuth. 1992. Fecal NIRS equations for predicting diet quality of free- ranging cattle. *J. Range Manag*. 45:238. doi:10.2307/4002970.

Merchen, N. R. 1988. Digestion, absorption and excretion in ruminants. In: Church., D. C., editor. *The ruminant animal: digestive physiology and nutrition*. Englewood Cliffs, NJ, USA: Prentice Hall; p. 172–201.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. *J. AOAC Int*. 85(6):1217–1240. https://academic.oup.com/jaoac/article/85/6/1217/5656649.

Moore, K. J., and L. E. Moser. 1995. Quantifying developmental morphology of perennial grasses. *Crop Sci*. 35:37–43. doi:10.2135/cropsci1995.0011183x003500010007x.

Norris, K. H., R. F. Barnes, J. E. Moore, and J. S. Shenk. 1976. Predicting forage quality by infrared reflectance spectroscopy. *J. Anim. Sci*. 43:889–897. doi:10.2527/jas1976.434889x.

Oba, M., and M. S. Allen. 1999. Evaluation of the importance of the digestibility of neutral detergent fiber from forage: effects on dry matter intake and milk yield of dairy cows. *J. Dairy Sci*. 82:589–596. doi:10.3168/jds.S0022-0302(99)75271-9.

Ovani, V. S., A. L. Abdalla, S. P. Márquez, W. dos S. da. Costa, B. E., Bizzuti, P. de M. T. Lima, G. D. Moreira, L. Gerdes, and H. Louvandini. 2022. Use of internal markers to estimate feed intake and selection of forage in sheep fed grass and legume hay. *Anim. Feed Sci. Technol*. 283:115177. doi:10.1016/j.anifeedsci.2021.115177.

Purnomoadi, A., M. Kurihara, T. Nishida, M. Shibata, A. Abe, and K.-I. Kameoka. 1996. Application of near infrared reflectance spectroscopy to predict fecal composition and its use for digestibility estimation. *Anim. Sci. Technol*. 67:851–861. doi:10.2508/chikusan.67.851.

Purnomoadi, A., M. Kurihara, T. Nishida, F. Terada, A. Abe, and T. Hamada. 1997. Two methods of near infrared reflectance spectroscopy for determining the digestibility and energy value of feeds. *Anim. Sci. Technol*. 68:351–359. doi:10.2508/chikusan.68.351.

Raffrenato, E., D. A. Ross, and M. E. van Amburgh. 2018. Development of an in vitro method to determine rumen undigested aNDFom for use in feed evaluation. *J. Dairy Sci*. 101:9888–9900. doi:10.3168/jds.2018-15101.

Righi, F., M. Simoni, G. Visentin, C. L. Manuelian, S. Currò, A. Quarantelli, and M. de Marchi. 2017. The use of near infrared spectroscopy to predict faecal indigestible and digestible fibre fractions in lactating dairy cattle. *Livest. Sci.* 206:105–108. doi:10.1016/j.livsci.2017.10.006.

Shenk, J. S., and M. O. Westerhaus. 1991. Population definition, sample selection, and calibration procedures for near infrared reflectance spectroscopy. *Crop Sci.* 31:469–474. doi:10.2135/cropsci1991.0011183x003100020049x.

Showers, S. E., D. R. Tolleson, J. W. Stuth, J. C. Kroll, and B. H. Koerth. 2006. Predicting diet quality of white-tailed deer via NIRS fecal profiling. *Rangel. Ecol. Manag.* 59:300–307. doi:10.2111/04-069.1.

Simoni, M., A. Goi, M. de Marchi, and F. Righi. 2021. The use of visible/near-infrared spectroscopy to predict fibre fractions, fibre-bound nitrogen and total-tract apparent nutrients digestibility in beef cattle diets and faeces. *Ital. J. Anim. Sci.* 20:814–825. doi:10.1080/1828051X.2021.1924884.

Stuth, J., A. Jama, and D. Tolleson. 2003. Direct and indirect means of predicting forage quality through near infrared reflectance spectroscopy. *Field Crops Res.* 84(1–2):45–56. doi:10.1016/S0378-4290(03)00140-0.

van Amburgh, M. E., R. J. Grant, K. W. Cotanch, A. Zontini, D. A. Ross, and A. Foskolos. 2015. NDF making something old, new again. In: Proc. Herd Health Nut. Conf.