# Applicability of near infrared reflectance spectroscopy to predict dry matter intake and neutral detergent fiber digestibility in mature geldings consuming an all-forage diet[1]

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**ABSTRACT:** An experiment was conducted to investigate the applicability of near infrared reflectance spectroscopy (NIR) to predict DMI and in-vivo NDF digestibility in horses. Nine mature, sedentary stock-type geldings were randomly assigned to one of three treatments within three simultaneous 3 x 3 Latin Squares to compare intakes and digestibilities of alfalfa (A), coastal Bermudagrass (C), and mixed grass hay (MGH). The 51-d study consisted of three 17-d periods. Each period consisted of a 4-d dietary adjustment period, 10-d feeding period, and 3-d total fecal collection period. There was a main effect of treatment (P = 0.02) on overall mean DMI. Horses consuming A had a greater mean DMI (P = 0.02) as compared to horses consuming MGH (7.31 vs. 5.41 kg/d). Overall mean DMI for horses consuming A and C (7.31 vs. 6.79 kg/d) were similar (P = 0.71). Dry matter intake was negatively correlated (r = -0.44) with NIR analysis of NDF with sulfite and ash correction (aNDFom). Dry matter intake was significantly (P = 0.02) related to hay aNDFom; however, the $R^2$ value was 0.17, which indicated that 17% of the variation in DMI could be explained by the aNDFom content of hay. Dry matter intake was negatively correlated (r = -0.45) with NIR analysis of undigested NDF at 30 h (uNDF30). There was a main effect of treatment on in-vivo NDF digestibility (P = 0.01). Correlations between in-vivo NDF digestibility and NIR hay analysis were not significant (P > 0.61) for aNDFom, uNDF30, or NDF digestibility at 30 h (NDFD30). Results from this study indicate that the use of NIR to estimate intake and in-vivo NDF digestibility in horses warrants further research. Perhaps the further collection of data will lead to development of NIR calibration equations that will be robust enough to predict NDF digestibility in horses.

Key Words: Equine, NDF digestibility, forage, NIR, in-vivo, in-vitro

**INTRODUCTION:** Traditional in-vivo methods of determining digestibility, while accurate, can be costly and time-consuming. As a result, in-vitro methods of determining digestibility have been developed. Utilizing in-vitro technologies often require a microbial inoculum prepared from ruminal or cecal fluid extracted from cannulated animals. This has proven to be a disadvantage and has limited the use of in-vitro methodologies in equine research, as cecally-cannulated horses are not readily available. The use of near infrared reflectance spectroscopy (NIR) been accepted as a useful tool for analyzing feeds and forages. The application of NIR to predict chemical composition and digestibility coefficients of forages has been observed in multiple species. Park et al. (1998) reported $R^2$ values of 0.85 for DM digestibility and OM digestibility, 0.79 for NDF digestibility, and 0.85 for GE digestibility for NIR to predict digestibility in sheep. Andrieu and Martin-Rosset (1995) observed r = 0.98 correlation between in-vivo OM digestiblity and OM digestibility prediction from NIR in horses. The NDF analysis provides an estimate of total cell wall constituents of forages, and is highly correlated with intake and rate of digestion in ruminants (Mertens, 1997). While nutritionists have been primarily concerned with fiber digestibility, recent efforts have focused on the concept of "undigestible NDF," (uNDF) as it sets the extent and rate of fiber fermentation in the rumen. According to Cotanch et al. (2014), uNDF should be included in routine forage analysis, as uNDF offers a more predictable digestibility and uniformity as compared to NDF. While data from several studies have successfully predicted digestibility with NIR in other species, research pertaining to the prediction of in-vivo NDF digestibility in horses are minimal. The objective of this study is to investigate

applicability of NIR to predict DMI and in-vivo NDF digestibility in mature geldings consuming an all-forage diet.

## MATERIALS AND METHODS

### *Horse Care and Management*

Nine mature, sedentary stock-type geldings, ranging from 10 to 20 yr of age were randomly assigned treatments within 3 simultaneous 3 x 3 Latin square design experiments to compare intakes and digestibilities of mixed grass hay (MGH), coastal Bermudagrass hay (C), and alfalfa hay (A). Horses were allowed 3 h to consume rations before being turned out into 6 x 20 m pens for both morning and evening feedings. Horses were fed hay initially at 3.0% BW/d for the first 2 d of the trial, and feed offered was adjusted on d 3 based on avg intakes. All horses in the study were classified under "Maintenance" according to the NRC (2007). Experimental protocol for this trial was approved by the WTAMU Institutional Animal Care and Use Committee.

### *Experimental Treatments*

The 51-d study consisted of three 17-d periods. Each period consisted of a 4-d dietary adjustment period, a 10-d feeding period, and a 3-d total fecal collection period. Nutrient analysis of hays are listed in Table 1. Hays were selected based on availability and differing sulfite and ash corrected NDF (aNDFom) concentrations. To meet Equine NRC (2007) requirements for CP and DE, 0.68 kg of ground soybean meal (Hi Pro 47.5% Bagged Soybean Meal, Hi Pro Feeds, Friona, TX, USA) was fed daily to horses receiving MGH.

***Hay Sample Collection and Preparation***

Prior to the study, treatment hay samples were collected using a Penn State Forage Sampler, (Scientific Systems, Inc., Ft. Atkinson, WI.). Samples were sent to Dairy One Forage Lab (Ithaca, NY) for analysis. During the fecal collection period, orts were weighed and a representative sample was taken per feeding. Composite orts samples were labeled by horse, time, period, and treatment. Ground hay samples were scanned using a Foss NIRSystems Model 6500 NIR with Win ISI II v1.5 (Foss NIRSystems, 7703 Montpelier Road, Suite 1, Laurel, MD 20723) to determine component concentrations.

***Analysis by Near Infrared Reflectance Spectroscopy***

Hay components that were analyzed by NIR included DM, CP, soluble protein (SP), RDP, Acid Detergent Insoluble Protein (ADI-CP), Neutral Detergent Insoluble Crude Protein (NDI-CP), ADF, amylase and sodium sulfite treated Neutral Detergent Fiber (aNDF), lignin, starch, Water Soluble Carbohydrates (WSC), Ethanol Soluble Carbohydrates (ESC), fat, ash, Ca, P, Mg, K, S, Cl, NDF Digestibility (NDFD) at 24, 30, and 48 h, starch digestibility, aNDF on an OM, ash free basis (aNDFom); undigestible NDF on OM, ash free basis at 30, 120, and 240 h (uNDFom); and NDF Digestibility on an OM, ash free basis at 30, 120, and 240 h (NDFDom).

***Fecal Analysis***

For dry matter determination, fecal samples were scanned using a Foss NIRSystems Model 6500 NIR with Win ISI II v1.5 (Foss NIRSystems, 7703 Montipelier Road, Suite 1, Laurel, MD 20723). Fecal samples were tested for NDF using the Filter Bag Technique (Method 6; ANKOM Technology, 2011). Following the NDF extraction,

the fiber residue was ashed for 450°C for 8 h to determine NDF on an ash-free, or OM, basis.

Three sets of 3 fecal samples were weighed individually at 0.5 g into 9 filter bags to determine uNDFom at 30, 120, and 240-h time points. Samples were subsequently analyzed with the addition of an ashing step to burn the undigested fibrous residue at 550°C for 2 h. Undigested NDF is expressed on an OM (ash free) basis as a percentage of the DM at 30, 120, and 240-h time points to reflect rates of undigested NDF digestibility.

***Statistical Analysis***

Data for DMIs and digestibilities were analyzed using the Mixed Procedure of SAS v. 9.4 (2014; SAS Institute, Inc., Cary, NC), with period and treatment as main effects. The effect of horse was included in the statistical model as a random effect. All means were compared using least squares means with a Tukey PDIFF adjustment to detect differences in treatment means. Simple correlations were calculated between DMI, in-vivo NDF digestibility, fecal aNDFom content, and NIR estimates using PROC CORR procedure of SAS. Prediction model data for DMI and NIR predictions were developed using PROC REG procedure of SAS. Significant differences were declared at $P < 0.05$, while trends were declared at $P < 0.10$.

## RESULTS AND DISCUSSION

Overall mean DMI and DMI as a percentage of BW for A, C, and MGH are listed in Table 2. It is important to note that in the current study, horses were not fed ad-libitum due to a restriction of time; therefore, DMI data may be confounded. There was a main effect of treatment ($P = 0.02$) on overall mean DMI. Horses consuming A had a greater

mean DMI ($P$ = 0.02) as compared to horses consuming MGH (7.31 vs. 5.41 kg/d). Overall mean DMI for horses consuming A and C (7.31 vs. 6.79 kg/d) were similar ($P$ = 0.71). There was a trend for horses consuming C to have greater ($P$ = 0.09) DMI than for horses consuming MGH (6.79 vs. 5.41 kg/d). There was a main effect of treatment ($P$ = 0.02) on overall mean DMI as a % of BW. Horses consuming A and C had a greater mean DMI as a percent of BW ($P$ = 0.01) as compared to horses consuming MGH (1.4 vs. 1.0% /kg BW, respectively). Dry matter intakes as a percentage of BW for horses consuming A and C (1.3 vs. 1.0% /kg BW) were similar ($P$ = 0.58). Results from the current study suggest that while NDF content of forage influences DMI in horses, other factors including palatability and individual preferences may also impact DMI.

Overall mean DM and NDF digestibilties for A, C, and MGH are shown in Table 2. There was a main effect of treatment ($P$ = 0.01) on overall mean DM digestibility. Horses consuming A had a greater overall mean DM digestibility ($P$ = 0.01) than horses consuming MGH (60.81 vs. 34.31%). Horses consuming A had a greater DM digestibility ($P$ = 0.04) than horses consuming C (60.81 vs. 49.60%). Horses consuming C had a greater DM digestibility ($P$ = 0.01) than horses consuming MGH (49.60 vs. 34.31%). There was a main effect of treatment ($P$ = 0.01) on NDF digestibility. Horses consuming C had greater ($P$ = 0.04) overall mean NDF digestibility as compared to horses consuming A (47.59 vs. 39.58%). Horses consuming C also had greater overall mean NDF digestibility ($P$ = 0.02) as compared to horses consuming MGH (47.59 vs. 38.50%). The lower NDF digestibility of A compared to C in the current study could be explained by differences in rate of passage, as a greater retention time increases digestibility (Mertens and Ely, 1982). Passage rate is influenced by water holding

capacity of the plant (Weyenberg et al., 2006). It was observed that horses consuming B had greater water consumption than horses consuming A. Grass hay has a greater hemicellulose content and absorbs more water than alfalfa (Cuddeford et al., 1992). Treatment C contained more hemicellulose and absorbed more water, which possibly slowed rate of passage; therefore, NDF digestibility increased. There were no differences in overall mean NDF digestibility ($P = 0.94$) between horses consuming A and horses consuming MGH (39.58 and 38.50%). The observed differences in NDF digestibilities of the grasses in the current study may be attributed to the differences in quality and NDF content of C and MGH. The CP content (3.7%) and NDF with a sulfite and ash correction (aNDFom) content (71.4%) of the MGH indicated an extremely low quality forage.

Correlations between DMI and NIR hay analysis are listed in Table 3. Dry matter intake was negatively correlated ($P = 0.02$) with aNDFom (r = -0.44) and undigested NDF at 30 h (uNDF30) (r = -0.45), indicating that as aNDFom and uNDF30 of the hay increased, DMI decreased. Dry matter intake was positively correlated ($P = 0.01$) with in-vitro NDF digestibility at 30 h (NDFD30) (r = 0.50), which suggest that DMI increased as NDF digestibility increased. Future research is needed to determine the effect uNDF has on fermentation to reach a better understanding of fiber digestion rates in horses.

Prediction equations using simple regression for DMI in relation to NIR hay analysis are listed in Table 4. Dry matter intake was significantly ($P = 0.02$) related to aNDFom with $R^2 = 0.17$, which indicated that 17% of the variation in DMI was explained by aNDFom content of hay. Dry matter intake was significantly ($P = 0.02$) related to hay uNDF30 with $R^2 = 0.17$, which indicated that 17% of the variation in DMI was explained by uNDF30 content of hay. Differences in particle retention time for grasses vs. legumes

can cause predicted uNDF30 to not correspond to actual uNDF30 (Jones and Siciliano-Jones, 2014). Because legumes have a lower cell wall content and a greater rate of passage, the use of alfalfa in the current study possibly impacted these results.

Correlations between in-vivo NDF digestibility, aNDFom content of feces, and NIR predictions from hays are listed in Table 3. Correlations between in-vivo NDF digestibility and NIR hay analysis were not significant ($P > 0.61$) for aNDFom, uNDF30, or NDF digestibility at 30 h (NDFD30). Overall, the hay NIR analyses were poor predictors of in-vivo NDF digestibility. Correlations between aNDFom content of feces and NIR predictions from hays were significant ($P = 0.01$) for aNDFom, uNDF30, and NDFD30. Correlation coefficients were r = 0.79 for aNDFom, r = 0.78 for uNDF30, r = -0.68 for NDFD30. The data suggest that as aNDFom and uNDF30 content of hay increased, aNDFom content of feces also increased. The data also suggest that as NDF digestibility increased, NDFom content of feces decreased.

To apply the data in the current study in the field, perhaps the development of NIR calibration equations will improve the accuracy of NIR to predict NDF digestibility in horses. Equation development would require the construction of an extensive database using hundreds or thousands of samples, and would take several years to implement. As uNDF30 content of forage contributes to intake in horses, further research is warranted to develop an NIR equation using uNDF30. This could help direct equine nutritionists toward a better understanding of the relationship between forage quality and intake.

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***Tables and Figures***

Table 1. Nutrient analysis of alfalfa, coastal Bermudagrass, and mixed grass hay (DM Basis).

| Item, Unit | Alfalfa | Coastal | Mixed Grass |
|---|---|---|---|
| DM, % | 89.7 | 90.7 | 92.9 |
| CP, % | 22.7 | 6.9 | 3.7 |
| ADF, % | 28.7 | 38.2 | 49.1 |
| aNDFom, % | 34.0 | 64.0 | 71.4 |
| Lignin, % | 6.9 | 6.4 | 6.2 |
| NFC, % | 30.6 | 20.2 | 14.4 |
| Ash, % | 10.20 | 6.56 | 8.75 |
| Ca % | 1.49 | 0.35 | 0.34 |
| P % | 0.28 | 0.19 | 0.04 |
| DE (Mcal/Kg) | 2.89 | 2.40 | 2.18 |
| %uNDFom 30hr | 20.5 | 39.5 | 44.8 |
| %uNDFom120hr | 18.7 | 27.8 | 23.8 |
| %uNDFom 240hr | 17.9 | 25.7 | 23.3 |
| | | | |
| %NDFDom 30hr | 39.8 | 38.3 | 37.2 |
| %NDFDom120hr | 45.0 | 56.6 | 66.7 |
| %NDFDom 240hr | 47.5 | 59.9 | 67.4 |

[a] Undigested NDF on an ash free, OM basis at 30 h
[b] Undigested NDF on an ash free, OM basis at 120 h
[c] Undigested NDF on an ash free, OM basis at 240 h
[d] NDF Digestibility on an ash free, OM basis at 30 h
[e] NDF Digestibility on an ash free, OM basis at 120 h
[f] NDF Digestibility on an ash free, OM basis at 240 h

Table 2. Effect of treatment on mean body weight (BW), body condition score (BCS), dry matter intake (DMI), DMI (%BW), DM digestibility (DMD; %), and NDF digestibility (NDFD; %) in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

| | Alfalfa | Coastal | Mixed-grass | SEM | *P*-value |
|---|---|---|---|---|---|
| Average BW, kg | 508 | 509 | 502 | 27.0 | 0.95 |
| BCS | 5.0 | 5.0 | 6.0 | 0.25 | 0.23 |
| DMI, kg/d | 7.31[b] | 6.79[d] | 5.41[a,c] | 0.65 | 0.02 |
| DMI, % BW | 1.4[b] | 1.3[b] | 1.0[a] | 0.13 | 0.02 |
| TDMD, % | 60.81[c] | 49.60[b] | 34.31[a] | 4.17 | 0.01 |
| NDFD, % | 39.58[a] | 47.59[b] | 38.50[a] | 3.15 | 0.01 |

[a] Means without common superscripts differ ($P < 0.05$).
[c,d] Means without common superscripts differ ($P < 0.10$).

Table 3. Simple linear correlations between dry matter intake, in-vivo NDFD, aNDFom content of feces, and NIR hay analysis in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

| | DMI | IVNDFD | aNDFomfeces | aNDFom | uNDF30 | NDFD30 |
|---|---|---|---|---|---|---|
| DMI | 1.0 | - | - | -0.44* | -0.45* | 0.50* |
| IVNDFD | - | 1.0 | - | 0.10 | 0.09 | 0.02 |
| aNDFomfeces | - | - | 1.0 | 0.79* | 0.78* | -0.68* |
| aNDFom | -0.44* | 0.10 | 0.79* | 1.0 | 0.99* | -0.96* |
| uNDF30 | -0.45* | 0.09 | 0.78* | 0.99* | 1.0 | -0.97* |
| NDFD30 | 0.50* | 0.02 | -0.68* | -0.96* | -0.97* | 1.0 |

*Correlations declared significant ($P < 0.05$) when different from 0.

[a] aNDF reported on an ash free, OM basis

[b] Undigested NDF measured at 30 h in-vitro

[c] NDF digestibility measured at 30 h in-vitro

Table 4. Prediction of dry matter intake (kg/d) from aNDFom and uNDF30 (%) content of hay from NIR analysis in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

| | | | Slope | | Intercept | |
|---|---|---|---|---|---|---|
| Item | Equation | $R^2$ | SE | *P*-value | SE | *P*-value |
| aNDFom | y = -0.04x + 9.22 | 0.17 | 0.02 | 0.02 | 1.03 | <0.01 |
| uNDF30 | y = -0.07x + 9.15 | 0.17 | 0.03 | 0.02 | 0.99 | <0.01 |