ACCURACY OF DIET NUTRITIVE VALUE PREDICTIONS FROM A FECAL NIRS PROFILING SYSTEM FOR GRAZING BEEF CATTLE

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Chapter I

NUTRITIVE VALUE DETERMINATION OF GRAZED FORAGES BY RUMINANT ANIMALS: A REVIEW

Abstract

Determining the nutritional value of grazed forages by cattle is fundamental to nutrition research. Proper sampling method, collection, handling techniques is essential to obtain results that are valuable to the producer or researcher. Decisions affecting sampling technique is based upon the type of forage to be sampled, forage characteristics and results desired from the sampling process. Hand-harvested sampling offers ease of collection and low input costs. However, this method allows for an unavoidable technician bias. By allowing animals to harvest forage, many advantages can be seen including the removal of human bias from the process. Forage samples are selected naturally by the animal in the environment in which they are accustomed. An additional set of challenges are faced by allowing animals graze forage samples collection process. Problems involving salivary contamination, voluntary grazing, incomplete recovery of ingested forage, fasting times and obtaining a representative sample are obstacles that researchers face. Sample preparation of masticated samples in another area of concern for forage research. Drying methods and air-drying temperatures can have effects on final nutrient value of analyzed forages. Cellulose, hemicellulose and organic matter digestibility values can all be altered by sample handling procedures. The magnitude of variation is dependent on handling methods and forage types. The use of near-

infrared reflectance spectroscopy (NIRS) has the potential to assist researchers in grazed forage nutrient determination. Using fecal samples from grazing ruminants, research has shown that the nutrient composition of grazed forages can be predicted, with some level of accuracy, using NIRS technology. This level of accuracy is dependent upon forage type and location of the grazed forages.

Introduction

One basic problem facing nutritionists is the determination of the nutrient value of forages grazed by ruminants. Many times ruminants graze an unknown composition of range species. Determining nutritive value of selected forage diets, and making supplemental adjustments based upon these diets, often resembles more of an art than a science. Proper sampling methods and procedures are important for the success of forage nutrient determination. In recent years the use of near-infrared reflectance spectroscopy (NIRS) has been investigated to determine relationships between NIRS spectra and nutritive value of ingested forages. The objective of this review is to provide a current summation of past literature and new advances in the area of forage nutrient determination.

Review

Hand-Harvested Samples. Hand-clipping forages involves randomly selecting a quantity of forage from a pasture with no regard for cattle selectivity. Several sites in a pasture are hand clipped in order to achieve a representative sample. There are many factors that affect the number of samples that are

required to adequately sample the forage available. These factors are further complicated when sampling non-monoculture forages such as native rangelands. Determination of the adequate number of samples depends on: 1.) Type of forage being sampled, 2.) Characteristics of the forage that are to be measured, 3.) Accuracy and precision desired from the sampling process. The advantages of hand clipping include rapid sampling and small equipment requirement. Hand-harvested samples are free of contamination from saliva or the rumen environment (10). Several clipped samples can be taken in less time than it takes to prepare one fistulated animal for sample collection.

It has been shown that clipped samples differ in nutrient composition than grazed samples (11,19). Typically, forage components that are desired in higher concentrations such as crude protein are under-estimated by hand clipping forages when compared to samples grazed by animals. Furthermore, the opposite is true for undesirable diet components such as indigestible fiber. Differences in nutrient constituents between clipped and grazed samples were studied by Coleman and Barth (6). Diet crude protein levels were 4.7% and 3.3% higher for cattle grazing Fescue-Lespedeza and Orchardgrass-clover pastures over three years when compared with clip samples respectively. Acid detergent fiber (ADF) was 0.16% lower for diets selected by grazing animals versus clipped samples for Fescue-Lespedeza pastures over a three year period (6).

Hand-plucking forages differs from hand clipping in that only those parts of the plant are collected that are believed to represent the diet selected by the

animal. Ideally, animals are observed in their natural grazing environment and sample selection is based upon this observation. Otherwise, this subjective sampling technique is dependent on the sampling technician and their knowledge of available herbage and the behavior of the grazing animals.

Differences in hand-plucked and esophageal samples were studied by Campbell et al (5). Fistulated cattle were grazed for a period of 20 minutes in early morning and late afternoon. During the sampling period, hand-plucked samples were collected while observing what fistulated cattle were grazing. This procedure was repeated three times on Midland bermudagrass and once on native grass. Ash concentrations were significantly higher in fistula samples when compared to hand plucked samples. This was most likely due to ash contamination from saliva. Chemical concentrations were more variable for fistula samples when compared to plucked samples. Crude protein tended to be greater in fistula samples while nitrogen free extract in fistula samples were less. Significance of these findings were not consistent. There were no consistent trends for observed for either crude fiber or ether extract. Hand-plucking is the preferred hand collection method. However, neither hand collection method completely accounts for the selectivity of grazing animals.

Hand harvesting samples can be a quick and easy way to obtain an estimate of forage nutritive value. This type of sampling requires low inputs of time, labor and equipment. Accuracy of hand harvesting forage samples is dependent upon the type and characteristics of the forage type being sampled. When employing this type of sampling technique, one should be aware of biases

it encompasses. Hand harvested samples tend to differ in nutrient composition because there is no regard for cattle selectivity.

Sampling with Animals. Forage sampling by animals is favored over hand sampling because it introduces animal selection factors into this technique of diet determination. However with animal selection, additional challenges are encountered to determine nutritive value of grazed forages. Additional sampling variability is found using animal harvested samples due to variability linked to both the sampling process and human processing. Telford and workers (1975) studied these differences in clipped forage composition and forage samples collected using cows grazing Midland Bermuda grass. Differences in grazed samples vs. hand clipped samples varied by time of the year. Cows selected for diets higher in ash, cellulose lignin and acid detergent fiber. Gross energy and neutral detergent fiber was higher in hand clipped samples than for grazed samples. In a second trial conducted two months later, cows selected diets higher in ash, neutral detergent fiber, lignin, gross energy, crude protein and organic matter digestibility. Only cellulose was greater in hand clipped samples than grazed samples. A reduction in forage quality was seen in clipped samples over this period due to a lack of rainfall. This study shows that grazed samples and hand clipped samples are not only different, but vary in their differences due to changes in forage nutritive value.

It has long been believed that rumen evacuation technique reduces the selectivity of the animal (10). Sampling forage with rumen fistulated animals requires the entire contents of the rumen to be removed prior to sampling. The

rumen is then cleaned by hand and rinsed to reduce sample contamination. After the animal is allowed to graze, the sample is removed from the rumen and its original contents are placed back into the rumen (13). This process of removing and replacing rumen contents may cause more physiological disturbance to the animal compared to esophageally fistulated animals.

Rumen Fistula vs. Esophageal Fistula. Both esophageal and rumen fistula sampling accounts for much of the selectivity of the grazing animal. Longer collection times and larger sample size are associated with the rumen sampling procedure. Generally, rumen fistulas are more easily established, and rumen fistulated animals require less care and maintenance compared to esophageally fistulated animals. Additionally, rumen fistulated animals are better suited for a wider variety of research objectives. Disadvantages of the rumen fistula include increased time and labor requirements to evacuate and clean the rumen. Depression of digestibility is also seen when three or more collections are made weekly, and a possible decrease in selectivity because of an empty rumen (10). The esophageal fistula has become more popular for the purpose of collecting diet quality because of the disadvantages of the rumen evacuation method.

Techniques using fistulated animals are not perfect however, the extent in which fistula samples represent the actual diet are dependent on several factors: (1) loss of forage during sample collection, (2) animal contamination of the fistula sample and, (3) chemical changes from sample preparation (14). More specific causes of sampling error are salivary contamination, incomplete

sample recovery and obtaining a representative sample in a large pasture (10). Voluntary grazing can be a problem requiring fasting, however, it is unclear whether cattle are less selective after a fasting period.

Loss of Forage During Sample Collection. Concern involving the use of the esophageal fistula is incomplete recovery of ingested forage. The concern with incomplete recovery of forage samples is not collecting all of the sample the animal is consuming and thus changing the composition of the collected sample. Completeness of forage recovery is primarily due to particle size and fiber content of fed diets. Mechanical influences of the esophageal fistula being present during sampling and fistula size can also cause recovery problems. Fistulas tend to become plugged with a forage bolus when present during the sampling process. Known mixtures of herbage were fed to sheep fitted with esophageal fistulas by Grimes and Watkins (8). Forage recovery ranged from 53 to 73% however botanical composition was not altered. In a similar study, Campbell and workers (5) observed unsatisfactory organic matter recovery rates with clipped forages fed to cattle. They reported that organic matter recovery rates ranged from 26 to 81% for forages with esophageally fistulated cattle. Organic matter recovery rates were 26%, 34% and 81% for clipped native grass, clipped bermudagrass and long stemmed alfalfa hay respectively while using esophageally fistulated cattle. Campbell and workers attributed poor recovery rates to a small cannula (inside diameter 28.6 mm), and plugging of the cannula was the primary reason for low recovery rates. Collection time for the long stemmed alfalfa treatment was shorter than for other forages. All recovered

samples were higher in ash concentration compared to fed samples. Crude protein of recovered samples was 0.6% less for clipped bermudagrass and 2.7% more for clipped native grass. Clipped bermudagrass and native grass showed no significant differences in fiber concentrations in fed versus recovered forage.

Animal Contamination of the Fistula Sample. There is disagreement regarding the extent of salivary contamination in masticated esophageal fistula samples. Any differences in the composition of the masticated samples and forage seem to be within the limits of experimental error except for ash content (9). Nitrogen composition of masticated forage samples have been shown to be representative of the forage grazed. Galt and Theurer (7) reported that significant changes in nitrogen concentrations in masticated samples is not due to salivary nitrogen. These changes in nitrogen concentration are attributed to differences in diet. Minerals are the major concern in sample contamination because ash makes up to 95.6% of saliva on a dry matter basis (9). Consequently, ash concentration can increase from 1 to 4% in masticated samples compared to clipped forage due to saliva (14). Due to this ash contamination, researchers should strongly consider reporting samples high in ash, such as masticated samples, on an organic matter rather than on a dry matter basis.

Chemical Changes From Sample Preparation. Extrusa sample preparation technique represents further opportunity for variation in diet quality estimates. Acosta and Kothmann (1) examined differences in drying procedures using esophageal samples. Crude protein concentration, corrected for organic

matter loss was not different in freeze-dried compared to oven or air dried samples. Cellulose was greater for air-dried and oven-dried than freeze-dried samples. All freeze-dried samples contained more hemicellulose than oven and air-dried samples for bermudagrass.

In a more recent experiment, Broesder (4), investigated the influence of drying method on diet quality estimates from ruminal masticate samples. Samples were dried in a forced air oven at 60° C in drying trays at a depth of 3 cm and 1cm. A third group was lyophilized. Lyophilized samples were allowed to thaw at 25° C under a vacuum of 60 millitorr until dry. In vitro organic matter digestibility (IVOMD) values differed for dormant wheatgrass across all drying methods. Lyophilized samples had the greatest IVOMD values, with samples dried at 3 cm having the lowest digestibility values. The difference in lyophilized and 3 cm samples was 10.1% percentage unit reduction in IVOMD. There were no differences in IVOMD for actively growing wheat grass across drying methods (4).

Barth and researchers (2) reported differences in various legume and grass forage samples and the esophageal fistula samples from those forages. Ash, acid-detergent fiber and acid-insoluble lignin were significantly higher for legume and grass fistula samples compared to forage when samples were dried at 45° C. In vitro dry matter digestibility (IVDMD) of legume fistula samples was significantly less than forage. In vitro dry matter digestibility of grass did not differ significantly between forage and fistula samples. Ash was significantly

higher for both grasses and legume masticate samples that were oven dried at 45° C when compared to samples dried at 65° C.

Differences exist between drying methods for determining nutrient composition of grazed forages. In addition to this, the magnitude of the difference depends on the nutrient being observed. Nitrogen seems to be effected least amount by drying method. Cellulose, hemicellulose, digestibility and ash concentrations have varying degrees of variability between drying methods. Above all, it is important to treat all samples equally, in handling and drying, to ensure comparisons will be relative within a sample set.

Two trials were conducted to study the effects of fasting time on nitrogen content of esophageally collected forage samples (12). The first trial fasted sheep 0, 1, 3, 6 and 9 hours and found no difference in mean Nitrogen content of the extrusa. A second trial fasted sheep 0, 2, 6 and 22 hours, again with no difference in mean nitrogen content of the masticated forage.

Differences in masticate samples at the beginning and end of a thirty minute sampling period were compared (12). Mean nitrogen content of the extrusa organic matter was not significantly different from the beginning of the sampling period compared to the end of the sampling period.

Langlands (12) examined changes in diet composition of sample diets. Fistulated sheep were grazed for eighteen months and a second group of fistulated sheep was introduced into the pasture. After a ten day acclimation period for the second group, esophageal masticate samples were collected. Nitrogen concentration was higher for sheep grazing the pasture for eighteen

months compared to sheep grazing only ten days prior to sampling, 3.63% and 3.44% respectively. Diet digestibility was not different.

The effect of fasting time seems to be one factor of non-consideration effecting nutritive values of grazed forages. However, nitrogen concentration is the only major nutrient extensively studied for this consideration. Fasting time does not seem to effect nitrogen concentrations of grazed forages. Grazing behavior of sheep seems to be effected based upon the amount of time previously spent on sampled pastures.

Forage sampling by animal selection is favored over hand sampling because it introduces animal selection factors into this technique of diet determination. However with animal selection, an additional level of complexity is entered into to determine nutritive value of forages. Animals are unpredictable and involuntary grazing and limited sampling time can bring an end to the sampling process. Other problems that are encountered are increases in salivary contamination and incomplete recovery of grazed samples. Using animal sampling techniques involves a trade-off for the inclusion of animal selectivity for additional sampling variability.

Forage Nutritive Value Determination Using Near Infrared Reflectance Spectroscopy. Near-infrared reflectance spectroscopy (NIRS) has been used to predict the nutrient concentrations of forages. It is believed that, with NIRS, fecal samples from grazing cattle can also be used to predict the nutrient quality of grazed forage. Wet chemistry values from esophageal or

ruminal masticated forage samples, are used to develop and test prediction equations from fecal spectrophotometry data.

Lyons and Stuth (16) conducted five trials to compare the relation between fecal NIRS spectrophotometry data and adjusted in vivo values for diet crude protein concentration and organic matter digestibility. The Post Oak Savannah sites at the study location was dominated by little bluestem and brownseed paspalum. Regression was used to adjust in vitro organic matter digestibility to vivo values for digestible organic matter. Fecal samples were dried at 60° for 48 hours then ground through a 1mm screen using an Udy cyclone mill. Samples were then analyzed with a Pacific Scientific NIR Scanner. Regression equations were then formulated from this data.

NIRS predicted crude protein percent was regressed on the reference crude protein percentage to give an R^2 of 0.86. NIRS predicted digestible organic matter was regressed on reference digestible organic matter to give an R^2 of 0.80 (16). Precision for crude protein for both locations was higher than digestible organic matter, 0.86 and 1.65 standard error of validation (corrected for bias) respectively.

A validation experiment was done by Lyons et al (17), to further test the equations developed in the previous studies (16). Several trials were arranged on different pasture types. Four trials were conducted on native pastures and one fertilized gulf coast ryegrass pasture. Pastures ranged from 5.4 to 27.1% crude protein and 50.4 to 74.1% digestible organic matter. Consequently, crude protein and digestible organic matter values were beyond the range of Lyons

and Stuth (16). Fecal NIRS predictions for crude protein were 5.3% to 27.3% and 53.8% to 77% for digestible organic matter (17). Results for crude protein reveled an R^2 = 0.98, Standard Error of Prediction (SEP) = 0.49, intercept = -0.1and slope = 0.98. Digestible organic matter gave an R^2 = 0.87, SEP = 1.12, intercept = 2.4 and slope = 0.97.

Crude protein was overestimated by NIRS on old world bluestem and native range in a study conducted by Bogdhan (3). Diet quality values were predicted by the equations derived by Lyons and Stuth (16). Low end NIRS predictions of 5% corresponded to 2.5% wet chemistry crude protein values, while a high end NIRS estimate of 15% corresponded to 10% laboratory value. This differs with research conducted with native range by Pruitt et. al (18) which showed a significant ($P<.001$) under prediction of crude protein by NIRS (8.2% CP) when compared to actual forage values (10.0% CP). Predictions shown by Bogdhan (3) were higher for old world bluestem and tall grass prairie forage types. Crude protein NIRS predictions were shown to be more accurate for old world bluestem (R^2 = 0.65) than those of native range pastures (R^2 = 0.53).

Bogdhan (3) reported that NIRS predictions more closely tracked changes in digestible organic matter (DOM) than crude protein. The correlation of NIRS and actual laboratory values produced an R^2 of .47 and .54 for native range and old world bluestem, respectively. Near infrared reflectance spectroscopy underestimated DOM laboratory values when actual DOM was below 65%.

Different methods of sample rehydration have been tested to evaluate the effects on scanning results (15). Samples were oven dried and placed in a

desiccator and analyzed at 1, 4, 8, 24, 48 and 72 hours. After each scanning, samples were placed back into the desiccater until the next scanning period. After scanning was completed at 72 hours, samples were stored on the laboratory counter at ambient humidity. Crude protein concentration from samples kept in the desiccater remained relatively stable. However, values for the counter samples continued to increase through the 72-hour scanning. Statistical data is not available for this experiment because of a lack of observations, however, differences were substantial.

Near infrared spectroscopy analysis of fecal samples has shown limited promise to be a quick, precise and a possible method of analyzing grazed diets of ruminants. Crude protein and digestible organic matter can be precise in the setting in which equations were formulated. However, as shown by Bogdhan (3) and Pruitt et. al (18) predictions are not accurate in different regions than where prediction equations were developed. Validation is recommended before this technology is used. Equations must be developed or adjusted for different regions with differences soil, forage types, fertility and other environmental factors.

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Chapter II

ACCURACY OF DIET NUTRITIVE VALUE PREDICTIONS FROM A FECAL NIRS PROFILING SYSTEM FOR GRAZING BEEF CATTLE

Abstract

Native tall grass prairie and bermudagrass (Cynodon dactylon) pastures were sampled, and fresh fecal samples collected monthly in central Oklahoma. Paired fecal and diet samples were used to evaluate fecal near infrared reflectance spectroscopy (FNIR) predictions for crude protein (CP), digestible organic matter (DOM), fecal nitrogen (FN) and fecal phosphorous (FP). Four criteria were used to evaluate the accuracy of FNIR predictions of CP, DOM, FP and FP. These included the coefficient of determination, the intercept and slope from the regression equations and the percent difference between the FNIR prediction and reference values. Differences between these paired samples were calculated between FNIR and reference values as a percent of the reference value. These differences were used to determine if predictions were accurate, marginally accurate or inaccurate. The percent of FNIR predictions that were inaccurate were 64.9, 57.1, 13.8 and 83.1%, for CP, DOM, FN and FP respectively. In addition to FNIR evaluation, these diet and fecal samples were used to generate new calibration equations for predicting CP, organic matter disappearance (OMD) and FN. Validation results from calibrations explained 98, 90 and 81% of the variation for reference values for FN, CP and OMD, respectively. This would indicate that exclusively localized calibrations for FN, CP and OMD yield more accurate results.

(Key Words: Beef Cattle, NIRS, Forage, 5 total)

Introduction

For years, researchers have studied the changes in forage nutritive value in an effort to improve grazing management and supplementation strategies. Real-time diet nutritive value predictions are a useful tool to aid beef producers in making management decisions. Systems intended to predict grazed forage nutritive value must be timely, inexpensive and accurate. These criteria are necessary for such a management tool to be widely accepted by producers.

Brown et al (6) reported that near-infrared reflectance spectroscopy (NIR) serves as a accurate method to quickly analyze large numbers of forage samples to be used in extension advisory applications. These authors also reported that broad-based calibration equations for tropical forages predicted nutritive values with similar accuracy as species-specific equations. Early works by Brooks (5) studied the possibilities of predicting diet nutritive values of Alaskan elk using fecal NIR techniques. Brooks (5) concluded that FNIR can be used with accuracy and precision similar to laboratory techniques to predict chemical components of ingested diets.

Efforts by Lyons and Stuth (10) and Lyons et al. (11) have evaluated the use of fecal FNIR to predict CP and DOM using validation sample sets that are closely related to the equation calibration data set. Equations explained 63 to 93% of the variation for CP and 71 to 80% of the variation for DOM. Lyons et al. (11) collected data from one of the previous sites and used existing calibration

equations to estimated diet CP and DOM. Results from this study explained 98% of the variation for CP and 87% of the variation for DOM.

Research by Bogdahn (4), Pruitt et al. (14) and Andrae et al. (2) from different regions of the United States compared CP and DOM from independent data sets to FNIR predictions. In these studies, FNIR estimates of diet CP explained 15 to 61% of CP concentration in grazed diet samples and FNIR estimates of DOM explained 51 to 67% of reference sample DOM concentration. Because precision and accuracy of FNIR diet nutritive value estimates have been variable, our objective for this experiment was to evaluate the accuracy of fecal NIR profiling to predict diet CP, DOM, FN and FP from grazed forages commonly grazed in Oklahoma.

Materials and Methods

Sample Collection and Processing. Native tall grass prairie and bermudagrass (*Cynodon dactylon*) pastures were sampled at the Oklahoma State University Range Cow Research Center west of Stillwater, Oklahoma. Monthly samples from the bermudagrass location (N 36.10919°, W 097.25360°) were collected from May to September of 1998 and from April to November of 1999. Monthly grazed samples from the native prairie location (N 36.15217° W 097.27470°) were collected from July to September of 1998 and from April to December of 1999 and in January and February of the year 2000. Fecal samples were collected at the time of diet sampling on each collection date. Paired fecal and diet samples were used to evaluate FNIR predictions for crude

protein (CP), digestible organic matter of grazed diets and fecal concentrations of nitrogen (FN) and phosphorous (FP).

Esophageal surgeries were performed under general anesthesia by faculty of the College of Veterinary Medicine, Oklahoma State University. Fistulated animals were allowed to graze pastures for a minimum of 7d prior to sampling. No additional supplementation was provided at any time during the experiment. To insure adequate sampling of grazed pastures, fistulated animals were not allowed to graze 16h prior to sampling. Diet samples were collected at 1100. However, during summer months, when the 1100 temperature was expected to exceed 24 °C, samples were collected at 0700.

Grazed diet and fecal samples were immediately placed on ice until frozen following collection and stored at less than –3 °C. Fecal samples were divided into two aliquots and frozen. One aliquot was shipped on ice to the Grazing Animal Nutrition (GAN) Laboratory at Texas A&M University, College Station, Texas for FNIR spectral analysis. The second aliquot was retained at Oklahoma State University for chemical analysis. Grazed diet and fecal samples were thawed at 4 °C for 48h, and then dried in a forced air oven at 60 °C for 16 hours. Grazed diet and fecal samples were ground through a 2mm screen and stored in plastic bags for laboratory analysis.

Upon arrival at the GAN laboratory frozen fecal samples were dried at 60° in a forced air oven for 24h then ground to pass through a 1mm screen. Samples were then dried again for 12 h at 60° C to stabilize sample moisture (9) before scanning samples with NIRS instrument. Stabilizing moisture is important

because crude protein equations utilize wavelengths where protein and water absorption simultaneously occur (15). Samples were immediately placed in a desiccator for 1 hour to cool. Samples were removed from the desiccator, mixed thoroughly and tightly packed into cups equipped with a quartz lens. Samples were immediately scanned with a NIRSystems 5600 spectrophotometer (FOSS NIRSystems, Inc.; Silver Spring, MD). Diet predictions were then generated with fecal spectra using GAN lab equations for CP, DOM, Fecal N and Fecal P (15).

Chemical Analyses. Diet and fecal sample dry matter (DM) was determined by drying samples at 100° C for 24 hours. Organic matter (OM) concentrations of diet samples were determined as the weight loss during combustion in a muffle furnace at 500° C for 6 hours. Grazed diet and fecal nitrogen was analyzed by combustion method using a LECO (LECO-NS2000, Leco Corporation, St. Joseph, MI) instrument (3).

Forty-eight hour in vitro organic matter disappearance was used to predict in vivo organic matter disappearance using a modified method of Goering and Van Soest (7) as described by Ackerman et al (1). All in vitro analysis were conducted in triplicate. Digestible organic matter (DOM) was calculated by multiplying in vivo OM digestibility by sample OM and was expressed as a percentage of DM (1). Fecal phosphorus was determined by the procedure described by Verbeek (16).

These fecal and grazed diet samples were used to generate new calibration equations for the prediction of CP, organic matter disappearance (OMD) and FN. Calibration equations were developed using stored FNIR fecal

spectra (independent variable) and reference data generated from laboratory analysis (dependent variable). Calibration selection was based upon the relationship between the standard error of difference (SED) of laboratory analysis and the standard error of calibration (SEC). Standard error of calibration must be greater than the SED to avoid over fitting the data to the equation. However, the SEC must be less than 1.5 times that of the SED to eliminate those equations that are too broad for accurate prediction. The r^2 of calibration is percent of the variation in the reference values that is explained by FNIR predictions for the calibration procedure. Likewise, the r^2 of validation is the expected percent of variation in reference values that is explained if an independent sample set is used.

Statistical Analysis. Analysis of variance for means of bermudagrass and native tall grass prairie was performed and statistical differences between reference and predicted values were reported. Regression equations were used to evaluate the usefulness of FNIR profiling to predict grazed diet and fecal indices using the PROC REG procedure of SAS. Data were analyzed by regressing the observed reference values from chemical analysis (dependent variables, Y) on the corresponding FNIR derived estimate (independent variable, X). Slopes of the relationships between FNIR and reference values were evaluated to whether or not slope=1. A slope not significantly different from 1 with a high r^2 value will indicate a series of predicted data that is similar to the reference data. Predicted values derived from fecal FNIR are referred to from

this point forward as FNIR (i.e. FNIR CP), and reference values are referred to as CP, DOM, FN or FP.

In a final attempt to evaluate the accuracy of FNIR predictions, FNIR values were subtracted from reference values. This difference was expressed as a percent of the reference value (12) where the percent difference = ((Reference Value – FNIR Predicted Value) ÷ Reference Value) x 100. Predictions were deemed accurate if the predicted value was 95 to 105% (±5%) of the reference value. Marginally accurate predictions ranged from 90-95% and 105-110% (±5-10%) of the reference value. Inaccurate estimates were greater than or less than 10% different than the reference values.

Tables 1 and 2 show monthly precipitation and average temperature for the study period and 100-year average. In general, samples were collected under variable environmental conditions with the exception that late summer conditions for both years were warmer and dryer than normal (Tables 1 and 2). A summary of this data by species is presented in Table 3.

Results and Discussion

Crude Protein. When cattle grazed bermudagrass, FNIR mean for CP (Table 4) was 1.47 percentage units less than the mean CP value for reference data (P = 0.09) (Table 3). In contrast, FNIR diet crude protein estimates for cattle grazing native tall grass prairie tended to be greater than reference data (P = 0.10), with an average overestimation of 12.6%. Reference CP explained 51% of the variation in FNIR CP (Figure 1). Slope of the equation for crude protein significantly differed from 1 (P=0.01) and Y-intercept differed from 0 (P < 0.05).

According to this equation, FNIR CP tends to be greater than CP when FNIR CP is greater than 8.94%. Conversely, FNIR CP will tend to be less than CP when FNIR CP is less than 8.94%. Nine FNIR CP values (12.2%) fell within the limits selected for accurate predictions, while seventeen (22.9%) of the estimates fell within the marginally accurate range. The remaining 48 (64.9%) estimates fell outside of the chosen limits of accuracy.

Based on this equation, a FNIR CP value of 8 would correspond to a CP value of 8.22. According to NRC (13) this over-estimation of CP would result in a difference of 0.0 kg ADG, or 0.00 body condition score units difference in 60 days, assuming an 1100 pound Angus x Hereford crossbred cow in mid-gestation.

Sample range and mean for CP concentration of masticate samples were similar to those observed by Bogdahn (4) collected during similar times of the year. These authors reported stronger relationships (R^2 = 0.613) for FNIR CP of old world bluestem and native tall grass prairie. Reported slope and Y-intercept by Bogdahn (4) were 0.82 and –1.61 respectively. In the Bogdahn (4) work, FNIRS CP estimates were generally less than CP for the range of data reported.

Pruitt et al. (14) compared fecal FNIR CP to reference CP determined from esophageal native range diet samples in western South Dakota. Forage samples were collected during the months of December, March, April and May for two consecutive years. There was a significant difference between the overall means by month for FNIR CP ($P<0.001$). For each month evaluated, FNIR CP was less than CP. Differences between means by month for these data range

from 1.0% CP (P=0.1) to 3.4% CP (P<0.001). When differences were expressed as a percent of reference CP, fecal FNIRS underestimated monthly mean CP from 10.9 to 38.2 percent.

Andrae et al. (2) compared reference CP from clipped samples to FNIR CP predictions for cattle grazing tall fescue pastures in Georgia. These scientists found a linear relationship when CP concentration of clipped samples was regressed on FNIR CP (CP = 0.967 x FNIR CP; (r^2 = 0.15)).

It appears that when an independent data set is used, FNIR yields less accurate predictions for CP than for closely related sample sets. Over a range of samples these predictions can be over-estimated (4), under-estimated (14) or both as observed in this study. Fecal NIR spectral analysis does not appear to provide the consistent and accurate results required to be incorporated into a grazing management strategy.

Digestible Organic Matter. FNIR and DOM means did not differ for native tall grass prairie but were significantly different (P<0.01) for bermudagrass. However, the slope for the regression equation did not differ from 1. Reference DOM explained 32% of the variation in FNIR DOM (Figure 2). The range of DOM values were 2.8 times greater for reference DOM than for FNIR DOM (44.4 vs. 15.8). Thirteen FNIR DOM predictions (18.5%) fell within the limits selected for accurate predictions, while seventeen predictions for FNIR DOM (24.2%) fell within the marginally accurate range. Forty FNIR DOM (57.1%) data points fell outside the bounds of our chosen accuracy range.

Based on this equation, a FNIR DOM value of 54.1 would correspond to a DOM value of 47.8. According to NRC (13) this over-estimation of DOM would result in a difference of 0.55 kg ADG, or 0.92 body condition score units difference in 60 days, assuming an 1100 pound Angus x Hereford crossbred cow in mid-gestation. In this situation, the use of DOM values generated by this system would have greatly overestimated cow performance.

The range of DOM values for grazed diet samples from Oklahoma native range and old world bluestem was greater than those reported by Bogdahn (4). These researchers reported a coefficient of variation of 51% with slope and intercept was 0.632 and 24.19, respectively. Fecal NIR DOM over-estimated DOM when predicted values were greater than 65%.

Research conducted in Georgia (2) studied the relationship between DOM from clipped fescue samples and FNIR analysis. These authors reported that DOM accounted for 67% of the variability observed for FNIR DOM of clipped fescue samples. These results were notably higher than the results observed in the current study. These scientists found a linear relationship when DOM concentration of clipped samples was regressed on FNIR DOM (DOM = 1.01 x FNIR DOM; (r^2 = 0.67)). These authors selected fescue leaves only. It is possible that this practice may not fully account for animal selectivity during the collection process (8, 17).

Previously discussed research indicates that DOM can be more precisely (r^2 = 0.67) estimated compared to estimates of CP (r^2 = 0.15), while others indicate that CP estimates are more precise (r^2 = 0.61 vs. r^2 = 0.51). These

relationships are observed in general, when independent reference data are used.

Fecal Nitrogen. The relationship between FN and FNIR FN is shown in figure 3. Means for FN did not differ from FNIR FN for native tall grass prairie or bermudagrass. The slope did not significantly differ from 1 ($P>0.05$) the Y-intercept differed from 0 ($P<0.05$). Reference FN explained 89% of the variation observed by FNIR FN. Thirty-one predictions (47.7%) for FN were within the accurate range. Twenty-five predictions (38.5%) were deemed marginally accurate. Only 9 FNIR predictions (13.8%) were deemed inaccurate using this technique.

Predictions for FN appear to be a reliable means of determining the fecal nitrogen concentration of grazing cattle. The level of accuracy associated with FN predictions was far superior to that of CP or DOM. To date, there have been no other published works evaluating the effectiveness of FNIR to estimate FN.

Fecal Phosphorus. Means for FP did not differ for native tall grass prairie fecal samples. However, means for FP differed ($P<0.01$) for bermudagrass fecal samples. The relationship between FP and FNIR FP is shown in figure 4. Only 21% of the variation observed in FNIR FP was explained by reference FP. The slope was not equal to 1 ($P>0.10$) and the Y intercept differed from 0 ($P<0.05$). Fifty-four values (83.1%) for FP were deemed inaccurate. Seven predictions (10.8%) gave marginally accurate results for predicting FP. Only 4 predictions (6.15%) fell within the chosen limits of accuracy.

Predictions of FP using FNIR technology proved to be disappointing. This level of inaccuracy is intolerable for the prediction of phosphorous in feces of grazing cattle. To date, there have been no other published works evaluating the effectiveness of FNIR to estimate FN.

Calibration of new equations for this sample set generated promising results. Predictions for FN were the most successful yielding a calibration r^2 of 0.99 and validation 0.98. Calibration results for CP gave a r^2 of calibration and validation of 0.96 and 0.90 respectively. Organic matter disappearance (OMD) calibration equations yielded an $r^2 = 0.90$ for calibration and $r^2 = 0.81$ for expected validation results. Validation r^2 results for CP were similar, while calibration r^2 results for CP were superior to those reported in similar works (10). Calibration and validation r^2 results for OMD were similar to the results found for DOM reported in other works (10).

Implications

Area calibrations for predicting grazed diet values appear to be an accurate method of predicting the nutrient concentration of grazed of forages. Whenever limited sample sets are collected from the same location used to generate calibration equations, those relationships between predicted and reference values appear to be strong. However, when these same calibrations are applied to a range of forages and locations, it appears as if the strength of these predictions weakens. As previously stated, systems intended to predict grazed forage nutritive value must be timely, inexpensive and accurate. The most important of these, is accuracy. Near infrared reflectance fecal profiling

provides an acceptable level of accuracy for predicting nitrogen concentration of feces from grazing cattle. Fecal NIR profiling does not provide sufficient accuracy for predicting grazed diet nutritive value or phosphorous concentration of feces for cattle grazing central Oklahoma forages.

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TABLE 1. Monthly precipitation (inches) for study period and 100 year average; Stillwater, Oklahoma.

	Month											
Year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1998	3.66	0.47	7.10	5.22	3.34	1.38	1.80	1.50	4.59	8.72	5.68	1.53
1999	1.58	0.64	4.58	6.75	4.52	8.13	1.93	0.97	6.12	3.58	0.30	5.06
2000	0.90	1.12	-	-	-	-	-	-	-	-	-	-
Avg.	1.15	1.53	2.79	2.92	5.13	4.00	2.90	2.76	4.29	2.83	2.25	1.30

TABLE 2. Average Monthly Temperature (°F) for study period and 100 year average; Stillwater, Oklahoma.

	Month											
Year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1998	38.8	44.0	45.6	56.5	72.2	80.0	85.7	83.8	80.3	63.6	52.5	41.5
1999	39.1	48.6	47.5	60.3	67.7	75.2	82.7	84.0	70.1	61.2	56.0	42.9
2000	39.4	45.7	-	-	-	-	-	-	-	-	-	-
Avg.	33.6	38.6	48.2	59.3	67.7	76.2	81.6	80.3	72.1	60.5	48.5	37.4

TABLE 3. Bermuda and native tall grass prairie (NTGP) summary of reference data for CP, DOM, fecal N and fecal P (% of Dry Matter) used to evaluate FNIR.

Forage	Item	n	x	STD	MIN	MAX
Bermuda	CP	30	10.4	3.61	5.73	17.6
	DOM	30	57.4	8.36	39.2	74.7
	FN	32	1.57	0.37	1.07	2.26
	FP	32	0.51	0.19	2.02	0.89
NTGP	CP	44	7.77	2.56	3.41	13.9
	DOM	40	60.3	10.3	35.9	80.3
	FN	33	1.72	0.29	1.12	2.28
	FP	33	0.32	0.15	0.14	0.88

TABLE 4. Differences in reference and FNIR means for CP, DOM, fecal N and fecal phosphorous (% of Dry Matter).

Forage	Item	REF[a]	FNIR[b]	SEM[d]
NTGP[C]	CP[e]	7.77	8.75	0.42
	DOM	60.3	62.2	1.25
	FN	1.717	1.678	0.0537
	FP	0.3204	0.3642	0.0238
Bermuda	CP[e]	10.4	8.93	0.65
	DOM[f]	57.4	63.6	1.18
	FN	1.570	1.545	0.0642
	FP[f]	0.5085	0.2916	0.0287

[a]REF=Reference values
[b]FNIR=Predicted FNIR values
[c]NTGP= Native tall grass prairie
[d]Standard Error of the Mean
[e]Means differ P<0.10
[f]Means differ P<0.01

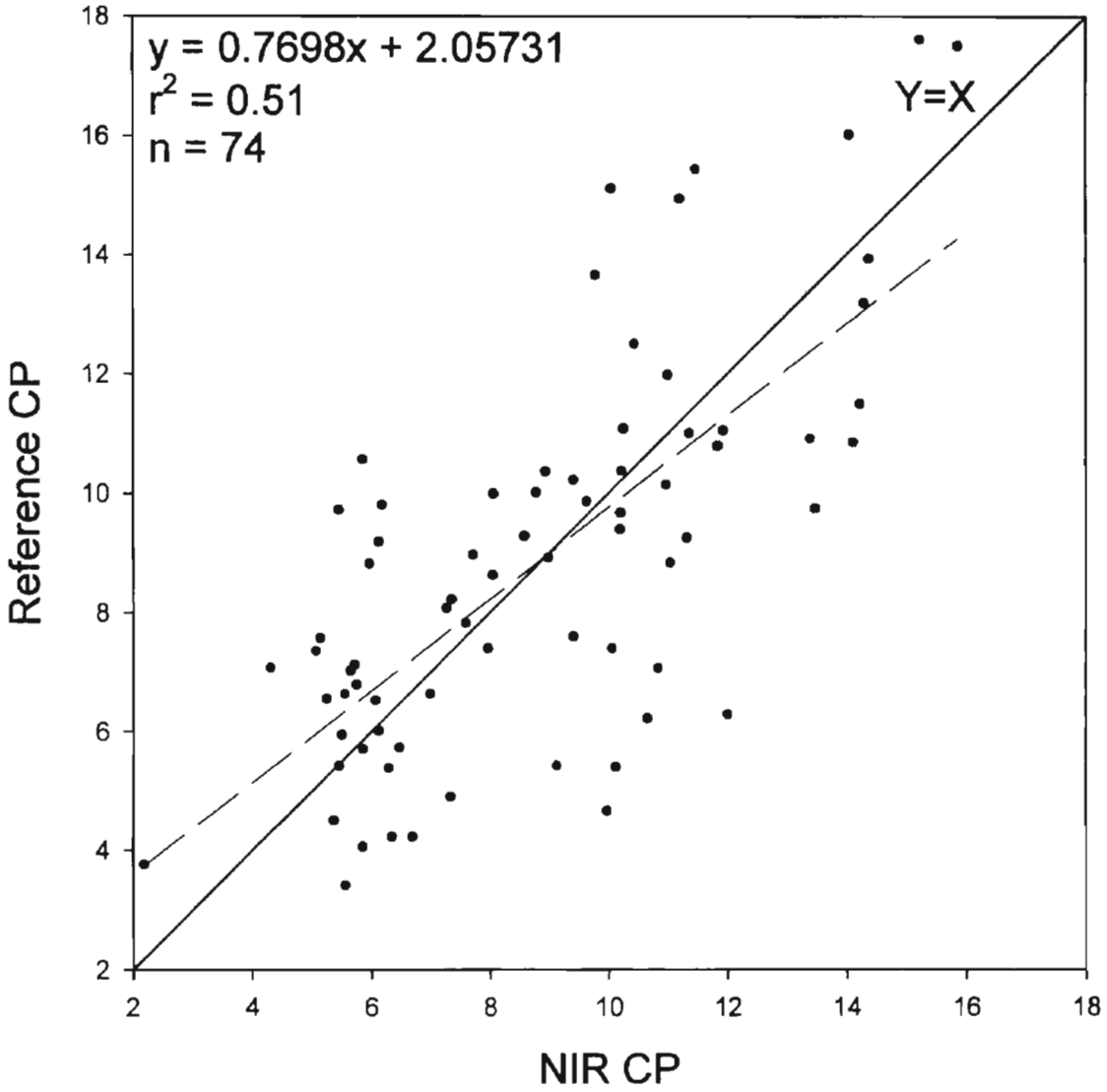

Figure 1. Relationship between reference CP and NIR CP

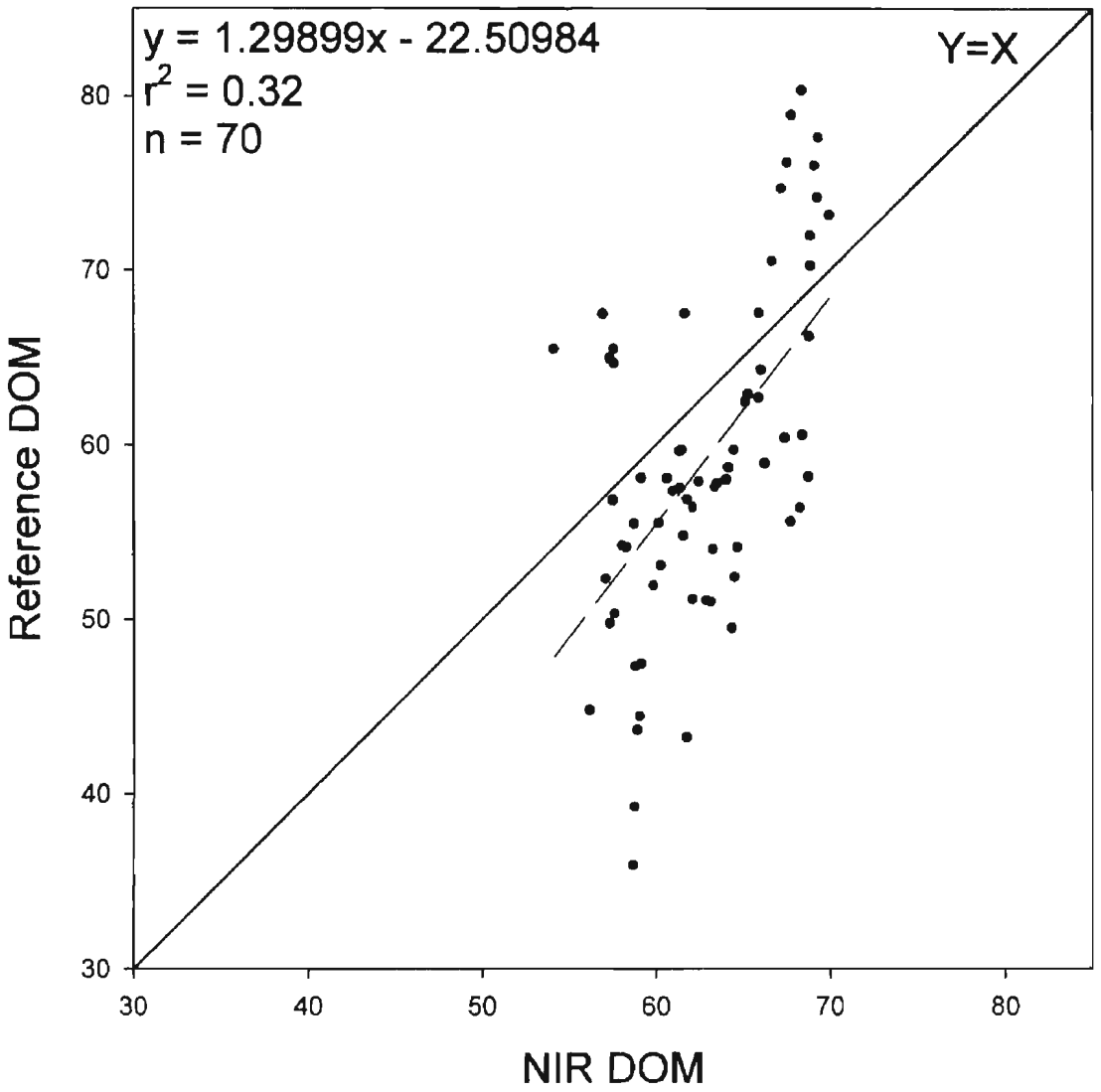

Figure 2. Relationship between reference DOM and NIR DOM

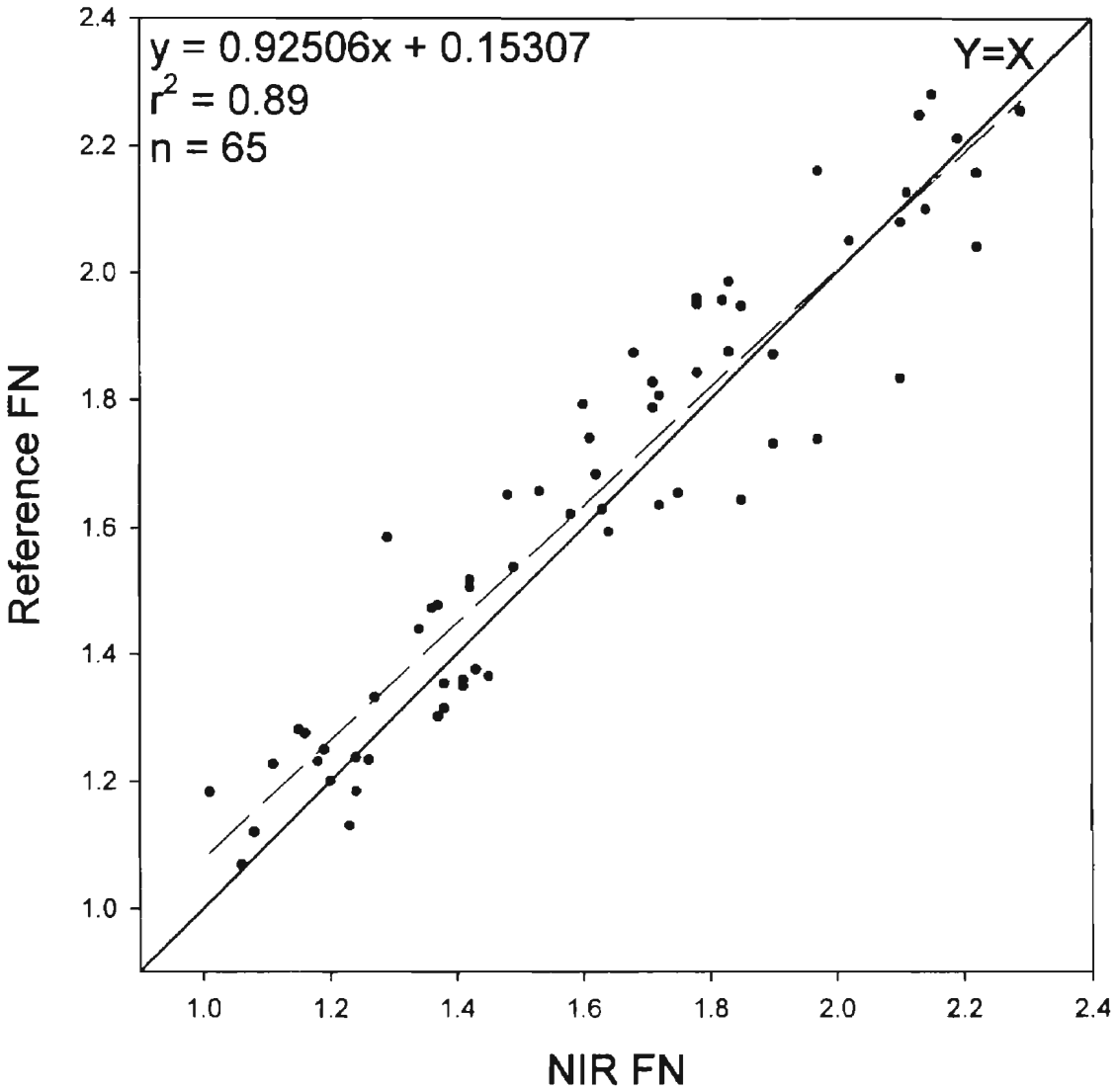

Figure 3. Relationship between reference FN and NIR FN

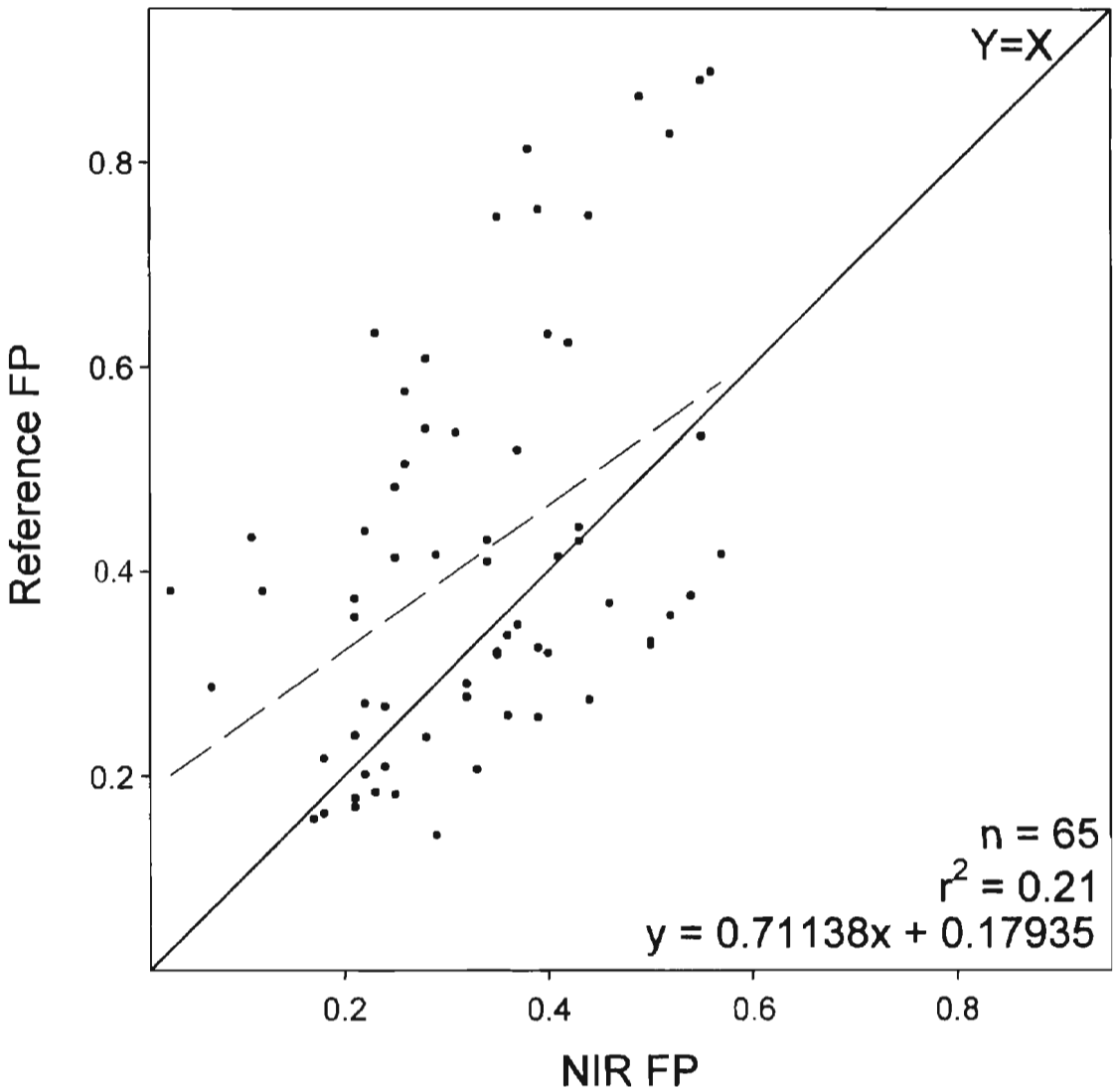

Figure 4. Relationship between reference FP and NIR FP

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