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Original Research Article

Accuracy of two optical chlorophyll meters in predicting chemical composition and *in vitro* ruminal organic matter degradability of *Brachiaria* hybrid, *Megathyrsus maximus*, and *Paspalum atratum*

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ABSTRACT

The objective of this study was to determine the accuracy and reliability of 2 optical chlorophyll meters: FieldScout CM 1,000 NDVI and Yara N-Tester, in predicting neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), acid detergent insoluble nitrogen (ADIN) and *in vitro* ruminal organic matter degradability (IVOMD) of 3 tropical grasses. Optical chlorophyll measurements were taken at 3 stages (4, 8 and 12 weeks) of regrowth in *Brachiaria* hybrid, and *Megathyrsus maximus* and at 6 and 12 weeks of regrowth in *Paspalum atratum* (cv. Ubon). Optical chlorophyll measurements showed the highest correlation ($r = 0.57$ to 0.85) with NDF concentration. The FieldScout CM 1,000 NDVI was better than the Yara N-Tester in predicting NDF ($R^2 = 0.70$) and ADF ($R^2 = 0.79$) concentrations in *Brachiaria* hybrid and NDF ($R^2 = 0.79$) in *M. maximus*. Similarly, FieldScout CM 1,000 NDVI produced better estimates of 24 h IVOMD ($IVOMD_{24h}$) in *Brachiaria* hybrid ($R^2 = 0.81$) and $IVOMD_{48h}$ in *Brachiaria* hybrid ($R^2 = 0.65$) and *M. maximus* ($R^2 = 0.75$). However, these prediction models had relatively low concordance correlation coefficients, i.e., CCC >0.90, but random errors were the main source of bias. It was, therefore, concluded that both optical chlorophyll meters were poor and unreliable predictors of ADIN and ADL concentrations. Overall, the FieldScout CM 1,000 NDVI shows potential to produce useful estimates of $IVOMD_{24h}$ and ADF in *Brachiaria* hybrid and $IVOMD_{48h}$ and NDF concentrations in *M. maximus*.

1. Introduction

Chlorophyll is responsible for the transitioning of radiant energy into chemical energy in plant green tissues (Gitelson & Merzlyak, 2003). The concentration of chlorophyll within green plants indicates its capacity to absorb radiant energy and hence its photosynthetic efficiency (Curran et al., 1990). Chlorophyll is, however, not uniformly distributed in the plant cell but confined to the chloroplast. In addition, chlorophyll concentration tends to be higher in young, more digestible leaves compared with the more fibrous mature leaves (Madakadze et al., 1999). Fibre and lignin are

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components of the structural fraction of the plant cell that maintain the structural integrity of the cell. These structural components do not contain chlorophyll, therefore, increasing the proportion of fibre and lignin dilutes the concentration of chlorophyll and hence indicates the quantity of light absorbed by the leaf. This presents the possibility of estimating fibre and lignin concentrations as well as forage degradability parameters if chlorophyll content is known. Indeed, Starks et al. (2006) suggested that the amount of light reflected or absorbed by a tropical grass canopy is partly dependent on the biochemical composition of plant tissues. This suggestion was earlier supported by Starks et al. (2004) who reported that a hand-held hyperspectral spectroradiometer accounted for 63%–79% variability of Bermuda grass (*Cynodon dactylon* L.) NDF and ADF concentrations. Albayrak (2008) reported R^2 values of the order of 0.74 and 0.81, respectively from NDF and ADF prediction models using a portable spectroradiometer in a sainfoin *(Onobrychis sativa* Lam.*)* sward. On the other hand, Hughes et al. (2014) reported very poor and unreliable estimates of ADF and lignin concentration of *Bracharia decumbens* pastures from optical chlorophyll measurements using the hand-held FieldScout CM 1,000 NDVI. The limited range within the examined parameters used in this later study was cited as a possible contributing factor to the poor relationships.

Detergent extraction methods and solubilization of cellulose with 72% sulphuric acid (Van Soest et al., 1991) are methods commonly used to analyse different fibre fractions and lignin concentrations of forage plants. Acid detergent insoluble nitrogen (ADIN) is determined by a two-step process that includes analysing for ADF followed by N determination on the residue. Measurement of forage degradability *in vitro* is also critical for accurate assessment of the nutritive value of forages. However, this procedure is costly and time-consuming and requires a well-equipped laboratory with highly skilled technicians in addition to the contentious requirement for surgically modified animals to provide rumen inoculum. Therefore, accurate, inexpensive and easy to use alternatives to the laboratory analytical methods will find favour with scientists, farmers and animal welfare advocates alike. Remarkably, there are no previous reports describing relationships between ADIN, *in vitro* ruminal organic matter degradability and optical chlorophyll measurements. Even less is known of the ability of the FieldScout CM 1,000 NDVI and Yara N-Tester to predict fibre, lignin and organic matter degradability of *Brachiaria* hybrid, *Megathyrsus maximus* and *Paspalum atratum*. The *Brachiaria* hybrid, in recent times, has grown significantly in popularity among livestock farmers in the Caribbean region. It is a semi-erect perennial tropical grass with vigorous growth and high tiller density. *M. maximus*, commonly known as Guinea grass, is a tall and erect perennial tropical grass. Historically, it is one of the more popular grass species within the Caribbean. *P. atratum* is a semi-erect perennial grass that is not common in the Caribbean but possesses great potential because of its high tiller density and leaf proportion. This experiment, therefore, seeks to determine the accuracy and reliability of the FieldScout CM 1,000 NDVI and Yara N-Tester to predict NDF, ADF, ADL, ADIN, and *in vitro* ruminal organic matter degradability of *Brachiaria* hybrid (cv. Mulato II), *M. maximus* (cv. Mombasa) and *P. atratum* (cv. Ubon).

2. Materials and methods

2.1. Establishment and management of grass species

Brachiaria hybrid cv. Mulato II (*Bracharia ruziziensis* × *B. decumbens* × *Bracharia brizantha*), *M. maximus* cv. Mombasa and *P. atratum* cv. Ubon were established from seeds. These seeds were sown in plastic seedling trays with a commercial potting mix as the rooting medium and kept under a greenhouse. Seedlings were manually irrigated daily using a watering can. A water-soluble liquid foliar fertilizer (20-20-20 NPK + trace elements) was diluted at a rate of 2.5 mL/L and applied at 3–5 days interval. Seedlings were transplanted at 5 weeks maturity in 17,663 cm^3 (diameter = 30 cm, height = 25 cm) cylindrical plastic pots filled with top soil of the St. Augustine series. One seedling was planted in each pot. The chemical and physical characteristics of the St. Augustine series were previously reported by Edwards et al. (2012). These pots were placed in an open-field at the University of the West Indies Field Station (10°38′15″N, 61°25′39″W) for the duration of the experiments (April–August, 2014). Mean monthly rainfall and daylight temperatures during the experimental period ranged 4–98 mm and 27–28.5 °C, respectively. Granular fertilizer was applied by band placement in each pot at transplanting, at a rate of 25 kg N, 18 kg P_2O_5 and 30 kg K_2O per hectare (1 ha = 10,000 m^2). The grasses were allowed to grow and cut back at 8 weeks of maturity to leave a 10 cm stubble height before the start of the experiment. Pots were randomly allocated to different treatment groups by grass species and fertilizer N in a 3 (stages of maturity regrowth, except for *P. atratum* that was harvested at 2 stages of regrowth) × 4 (N fertilizer applications) factorial arrangement. Each treatment had 3 replicates.

The treatments imposed were not to test their effects on chemical composition and *in vitro* ruminal fermentation parameters but rather to ensure adequate range in chemical composition and *in vitro* degradability parameters that will be used to develop prediction models.

2.2. Optical chlorophyll measurements

2.2.1. FieldScout CM 1,000 NDVI

The FieldScout CM 1,000 NDVI was developed by Spectrum Technologies Inc (360 Thayer Court, Aurora, IL 60,504) to measure chlorophyll concentration in green leaves. It utilizes laser directed "point and shoot" technology to rapidly measure light transmittance in the red (660 nm) and near-infrared (840 nm) spectral bands. Six optical chlorophyll measurements were taken from the canopy in each pot with the FieldScout CM 1,000 NDVI, and the average was calculated to represent the chlorophyll content of each pot. The FieldScout CM 1,000 NDVI was operated manually by holding it approximately 40 cm from the grass canopy at a 40–45° vertical angle. The laser was focused at different heights within the area to be harvested. FieldScout CM 1,000 NDVI measurements were taken by the same operator on all occasions prior to cutting for laboratory analysis.

2.2.2. Yara N-Tester

Yara N-Tester is a customized version of the Minolta Single Photon Avalanche Diode (SPAD-502) chlorophyll metre developed by Yara International (Hanninhof35 D-48249 Duelmen Germany) to assist with fertilizer recommendations in cultivated crops based on chlorophyll concentrations (Ortuzar-Iragorri et al., 2005).

It is equipped with 2 light emitting diodes and 1 silicon photodiode to measure light transmittance through green plant tissues at the red (650 nm) and near-infrared (960 nm) wavelengths within a 6 mm^2 area. Yara N-Tester produces a running average of 30 chlorophyll measurements for each reading. Six optical chlorophyll measurements were taken with this device from at least 5 leaves within each pot, and the average calculated to represent the chlorophyll measurement of each pot. The sensor of the Yara N-Tester was placed at the upper, middle and lower leaf blade of the grass to ensure the average reading was representative of the grass being sampled. Optical chlorophyll measurements with the Yara N-Tester were taken by the same operator on all occasions prior to cutting for laboratory analysis.

2.3. Grass sampling and sample preparation

Each pot represented an experimental unit, which was replicated 3 times per treatment. The treatments were arranged in a 4 (N fertilizer) × 3 (stage of maturity, except for *P. atratum* that was sampled at 2 stages of maturity) factorial design in a completely randomized design for each grass species. Grass sampling was done with a sharp knife at 4, 8, and 12 weeks of regrowth for *Brachiaria* hybrid and *M. maximus*. *P. atratum* samples were taken at 6 and 12 weeks of regrowth. *Brachiaria* hybrid and *P. atratum* were cut to leave a 15-cm stubble while a 20-cm stubble was left standing after the *M. maximus* was harvested. All herbage within the pot was cut and then sub-sampled for laboratory analysis at the Animal Nutrition Laboratory of the Department of Food Production, University of the West Indies, St. Augustine. Samples were placed in stainless steel oven pans and placed in a force-draft oven set at 65 °C and dried to constant weight.

After drying, samples were ground in a stainless-steel hammer mill (Thomas Wiley Laboratory mill, model 4; Thomas Scientific USA) to pass through a 1 mm sieve in preparation for chemical analysis. Ground samples were temporarily stored in air-tight ziplock bags pending chemical analysis.

2.4. Chemical analysis

The analyses of NDF, ADF and ADL were done sequentially using the filter bag technique in the ANKOM^{2000} Fibre Analyzer (model: A2000I) (ANKOM Technology, Macedon NY). Sodium sulphite and α-amylase were included in the NDF analysis. Both NDF and ADF were expressed inclusive of residual ash. Subsequent to ADF determination, ADL concentration was determined by solubilisation of cellulose with 72% sulphuric acid as described by Van Soest et al. (1991). Dried ADL residue was ignited in a muffle furnace at 550°C until completely ashed. The analysis of ADIN was done by N analysis of the ADF residue from the second set of samples. Nitrogen in the dried ADF residue was determined using the copper catalyst Kjeldahl method (AOAC, 2005 method; 976.05).

2.5. In vitro ruminal organic matter degradability

In vitro ruminal organic matter degradability (12, 24 and 48 h) was determined using an ANKOM DAISY^{II} incubator following the procedure for *in vitro* true degradability (ANKOM, 2001) method number 3. Rumen inoculum was provided by an adult male Barbados Black Belly sheep fitted with a rumen fistula.

The daily diet of the donor animal included *ad libitum* supply of freshly cut Tanner grass *(Brachiaria arrecta)* supplemented with approximately 0.5 kg commercial concentrate (140 g/kg crude protein) with free access to clean water and mineral blocks. The collection was performed at approximately 07:30 before the morning feeding in pre-warmed thermos flasks. The inoculum was prepared by filtering through multiple layers of cheesecloth. Microbes that are closely attached to the rumen digesta were added to the inoculum by blending approximately 500 g of fibrous rumen material at high speed. Samples sealed in ANKOM F57 fibre bags were placed in 4 incubator jars each filled with 1,600 mL of ANKOM buffer solution and placed in the incubation chamber. The temperature of the digestion jars was allowed to equilibrate at 39°C for 30 min prior to inoculation with 400 mL rumen inoculum. The headspace of each jar was purged with CO_2 gas to ensure anaerobic condition is maintained. Fibre bags were withdrawn at 12, 24 and 48 h post-inoculation and repeatedly rinsed with tap water until water became clear. *In vitro* organic matter degradation at 12, 24 and 48 h was determined as the loss of organic matter after washing, drying and ashing in a muffle furnace at 550 °C.

2.6. Statistical analysis and calculations

Pearson's correlation coefficients were used to test the linear association between optical chlorophyll measurements and chemical composition and IVOMD. Data normality was assessed using normal probability plots. Prediction models for the chemical composition and IVOMD were generated by analysing scatter plots subsequent to selection of the model that best fits the observed data. Optical chlorophyll measurements were entered as the independent variable. The prediction models were developed using the Excel statistical package (Microsoft office version 2007). Model significance was tested by ANOVA at significance level $P < 0.05$.

Detailed model evaluation was restricted to those models with a coefficient of determination (R^2) equal to or greater than 0.45. Concordance correlation coefficient (CCC) was used to simultaneously determine model precision (correlation coefficient estimate $-\ \rho$) and accuracy (bias correction factor $-\ C_b$) (Lin, 1989). The CCC analysis was conducted using MedCalc statistical software package version 14.10.2 (MedCalc Software bvba, Ostend, Belgium; http:www.medcalc.org; 2014). Mean square prediction error (MSPE) was also used to evaluate the efficiency of the prediction models. The MSPE was calculated as follows using the Model Evaluation System (MES) version 3.1.13 (Collage Station, TX; http://nutritionmodels.tamu.edu/mes):

$$\mathrm{MSPE} = \sum_{i=1}^{n} \frac{(O_i - P_i)2}{n},$$

where O_i and P_i represent observed and predicted means, respectively. The MSPE was further dissected into mean bias, regression bias and random error (Bibby and Toutenburg, 1977). Mean bias represented the mean difference between observed and predicted values. Regression bias estimated the error associated with the regression slope and random error represents the error not detected by the model.

3. Results

3.1. Descriptive statistics and correlation analysis

Descriptive statistics of optical chlorophyll measurements, chemical composition and IVOMD are presented in Tables 1 and 2, respectively. Mean NDF, ADF, ADL and ADIN values were highest in *P. atratum*. *Brachiaria* hybrid had the highest 48, 24 and 12 h IVOMD. Optical chlorophyll measurements were generally negatively correlated with NDF, ADF, ADL and ADIN (Table 3) except for *P. atratum* NDF and Yara N-Tester that returned positive correlation. Optical chlorophyll measurements had a positive correlation with IVOMD except for *P. atratum*. FieldScout CM 1,000 NDVI measurements had stronger linear associations with fibre fractions than the Yara N-Tester. This association was generally the highest with NDF concentration. The FieldScout CM 1,000 NDVI had the highest correlation with fibre fractions in *M. maximus* which ranged between −0.85 and 0.59. *In vitro* ruminal organic matter degradability for all the 3 incubation intervals had moderate to strong correlation with FieldScout CM 1,000 NDVI for *Brachiaria* hybrid ($r = 0.52$ to 0.75) and *M. maximus* ($r = 0.54$ to 0.83). Yara N-Tester also had moderate to strong correlation with IVOMD for all 3 incubation intervals of *M. maximus* ($r = 0.66$ to 0.77).

3.2. Relationship between optical chlorophyll measurements and chemical components

Polynomial regression models best represented the relationships between optical chlorophyll measurements and NDF

Table 1
Descriptive statistics of optical chlorophyll measurements and grass chemical composition.

Species/Parameter	Mean	N	SE	Min.	Max.	SD	CV, %
FieldScout CM 1,000 NDVI chlorophyll measurements							
Brachiaria hybrid	485	12	38.0	295	677	131	27.1
Megathyrsus maximus	388	12	37.3	176	568	129	33.3
Paspalum atratum	480	8	27.1	327	567	93.6	19.5
Yara N-Tester chlorophyll measurements							
Brachiaria hybrid	524	12	25.1	353	637	86.7	16.6
M. maximus	439	12	24.9	223	528	86.2	19.6
P. atratum	479	8	18.1	374	546	62.6	13.1
NDF, g/kg DM							
Brachiaria hybrid	537	12	15.6	446	624	53.9	10.1
M. maximus	627	12	9.6	591	691	33.2	5.3
P. atratum	634	8	3.2	619	647	9.1	1.4
ADF, g/kg DM							
Brachiaria hybrid	240	12	10.9	192	293	37.7	15.7
M. maximus	298	12	9.2	261	369	31.8	10.7
P. atratum	309	8	6.8	288	333	19.3	6.3
ADL, g/kg DM							
Brachiaria hybrid	18.9	12	1.23	14.2	30.0	4.30	22.6
M. maximus	31.8	12	4.30	16.7	62.7	14.8	46.4
P. atratum	36.2	8	3.70	23.4	52.6	10.4	28.6
ADIN, g/kg DM							
Brachiaria hybrid	0.32	12	0.02	0.23	0.42	0.06	19.5
M. maximus	0.40	12	0.02	0.30	0.53	0.07	18.4
P. atratum	0.55	8	0.01	0.39	0.95	0.18	32.1

N = number of observation (mean of 3 replicates); SE = standard error; Min. = minimum observation; Max. = maximum observation; SD = standard deviation; CV = coefficient of variation; NDF = neutral detergent fibre, ADF = acid detergent fibre; ADL = acid detergent lignin; ADIN = acid detergent insoluble nitrogen.

Table 2
Descriptive statistics of *in vitro* organic matter degradability (IVOMD) used to generate the regression models.

Species/Parameter	Mean	N	SE	Min.	Max.	SD	CV, %
48 h IVOMD							
Brachiaria hybrid	780	12	26.7	630	886	92.3	11.8
Megathyrsus maximus	684	12	26.3	558	811	91.6	13.4
Paspalum atratum	757	8	24.1	694	874	68.5	9.1
24 h IVOMD							
Brachiaria hybrid	652	12	22.0	549	784	76.2	11.7
M. maximus	560	12	18.5	454	673	64.1	11.5
P. atratum	571	8	18.2	479	628	51.5	9.0
12 h IVOMD							
Brachiaria hybrid	506	12	24.9	362	622	86.4	17.1
M. maximus	388	12	18.6	279	473	64.4	16.6
P. atratum	411	8	16.6	349	486	46.9	11.4

N = number of observation (mean of 3 replicates); SE = standard error; Min. = minimum observation; Max. = maximum observation; SD = standard deviation; CV = coefficient of variation; IVOMD = *in vitro* organic matter degradability (g/kg) post 12, 24 & 48 h incubation.

concentrations (Fig. 1). The FieldScout CM 1,000 NDVI had stronger relationships with NDF concentrations than the Yara N-Tester.

The coefficient of determination for NDF prediction models from FieldScout CM 1,000 NDVI ranged from 0.70 in *Brachiaria* hybrid to 0.79 in *M. maximus* ($P < 0.05$). The best Yara N-Tester NDF prediction model was observed in *M. maximus* ($R^2 = 0.72$; $P = 0.003$). Acid detergent fibre was best predicted in *Brachiaria* hybrid ($R^2 = 0.79$; $P = 0.000$) and *M. maximus* ($R^2 = 0.54$; $P = 0.005$) with the FieldScout CM 1,000 NDVI ($R^2 = 0.56$) (Fig. 2). Yara N-Tester gave poor predictions of ADF concentrations in all 3 species. Optical chlorophyll measurements were poor predictors of ADL (Fig. 3). In fact, the best ADL prediction was observed from the FieldScout CM 1,000 NDVI in *M. maximus* ($R^2 = 0.58$; $P = 0.005$). Optical chlorophyll measurements poorly predicted ADIN concentration. For example, the best ADIN prediction model was observed with the FieldScout CM 1,000 NDVI in *M. maximus* which only explained 39% ($P = 0.109$) of ADIN variation (Fig. 4).

3.3. Relationship between optical chlorophyll measurements and IVOMD

Optical chlorophyll measurements produced low to moderate $IVOMD_{48h}$ estimates (Table 4). The FieldScout CM 1,000 NDVI measurements accounted for the highest percentage of $IVOMD_{48h}$ in *Brachiaria* hybrid (65%; $P = 0.008$) and *M. maximus* (75%; $P = 0.001$). FieldScout CM 1,000 NDVI best predicted $IVOMD_{24h}$ in *Brachiaria* hybrid ($R^2 = 0.81$). The best $IVOMD_{12h}$ was observed in *Brachiaria* hybrid ($R^2 = 0.62$; $P = 0.013$). The best Yara N-Tester $IVOMD_{48h}$ prediction models were observed in *M. maximus* ($R^2 = 0.65$; $P = 0.002$) and *P. atratum* ($R^2 = 0.55$; $P = 0.138$).

The Yara N-Tester accounted for 53% of $IVOMD_{24h}$ variability in *M. maximus*. Both optical chlorophyll measurements poorly predict IVOMD in *P. atratum* for all incubation times ($R^2 = 0.22–0.55$). The best $IVOMD_{12h}$ Yara N-Tester prediction model was observed in *M. maximus* ($R^2 = 0.53$; $P = 0.036$).

3.4. Evaluation of selected prediction models

Variation between observed and predicted NDF, ADF and $IVOMD_{48h}$ was generally low (Table 5). The CCC was highest for ADF (0.88) and $IVOMD_{24h}$ (0.89) in *Brachiaria* hybrid, NDF (0.87) and $IVOMD_{48h}$ (0.86) in *M. maximus* and NDF concentration (0.83) in *P. atratum* from FieldScout CM 1,000 NDVI prediction models. Relatively high (≥0.87) *ρ and* C_b were observed from FieldScout CM 1,000 NDVI prediction models for *Brachiaria* hybrid ADF, $IVOMD_{24h}$ and $IVOMD_{48h}$, and *M. maximus* NDF and $IVOMD_{48h}$. Similarly, *ρ and*

Table 3
Correlation between optical chlorophyll measurements, chemical composition and *in vitro* organic matter degradability (IVOMD).

	FieldScout CM 1,000 NDVI			Yara N-Tester		
Species/Parameter	*Brachiaria* hybrid	*Megathyrsus maximus*	*Paspalum atratum*	*B.* hybrid	*M. maximus*	*P. atratum*
Fibre fractions						
NDF	−0.71*	−0.85**	0.57	−0.63*	−0.84**	0.74*
ADF	−0.76**	−0.75**	−0.32	−0.57	−0.38	0.18
ADL	−0.58	−0.73*	−0.21	−0.01	−0.27	0.25
ADIN	−0.45	0.59*	−0.38	−0.10	0.43	−0.50
IVOMD						
48 h	0.75**	0.83**	−0.23	0.48	0.77**	−0.65*
24 h	0.52	0.54	−0.53	0.40	0.66*	−0.31
12 h	0.73*	0.67*	−0.52	0.68*	0.72*	−0.52

NDF = neutral detergent fibre, ADF = acid detergent fibre; ADL = acid detergent lignin; ADIN = acid detergent insoluble nitrogen; IVOMD = *in vitro* organic matter degradability (g/kg) post 12, 24 & 48 h incubation.
*Significance at $P < 0.05$; **Significance at $P < 0.01$.

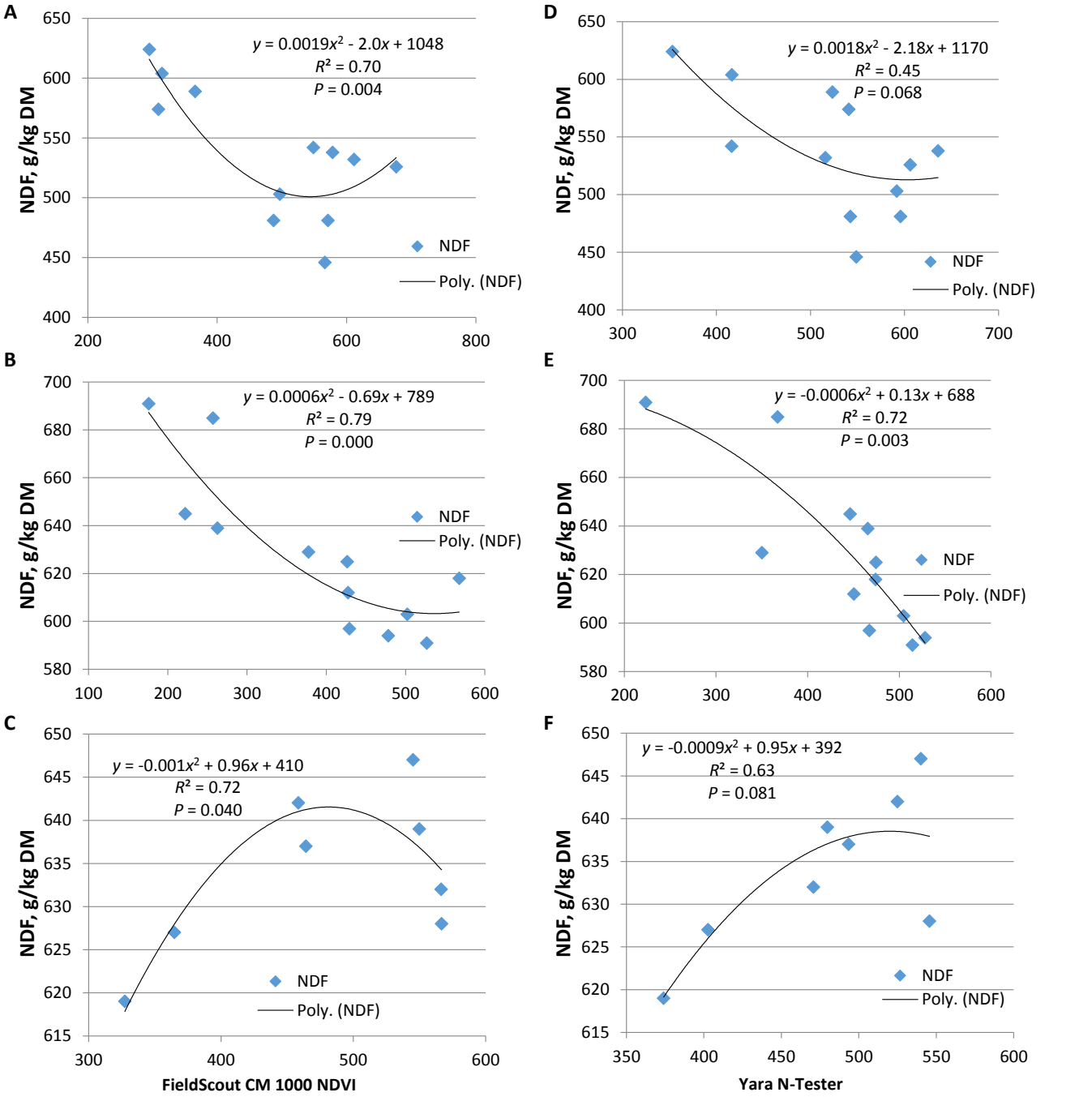

Fig. 1. Relationships between optical chlorophyll measurements and neutral detergent fibre (NDF, g/kg DM) concentrations of *Brachiaria* hybrid (A and D), *Megathyrsus maximus* (B and E) and *Paspalum atratum* (C and F).

C_b were relatively high in *M. maximus* Yara N-Tester NDF and $IVOMD_{48h}$ prediction models. The lowest MSPE corresponded with prediction models with the highest CCC for the respective species parameters. Random error was the primary source of error associated with the majority of the prediction models. Mean bias or regression bias was the highest with FieldScout CM 1,000 NDVI prediction models for *Brachiaria* hybrid NDF, *M. maximus* $IVOMD_{12h}$, ADF and ADL and *P. atratum* ADF. The proportion of random error of MSPE was highest for FieldScout CM 1,000 NDVI $IVOMD_{24h}$ (81.3%) in *Brachiaria* hybrid, for NDF (80.0%) in *M. maximus* prediction models.

4. Discussion

4.1. Relationship between optical chlorophyll measurements and chemical composition

Positive correlation between Yara N-Tester and *P. atratum* NDF concentration contradicts the expected outcome. The thick leaves and midribs of the *P. atratum* species could have negatively affected the Yara N-Tester measurements because it requires direct contact between the leaf surface and the metre sensor. Indeed, leaf and vein thickness has been previously acknowledged as plant factors that

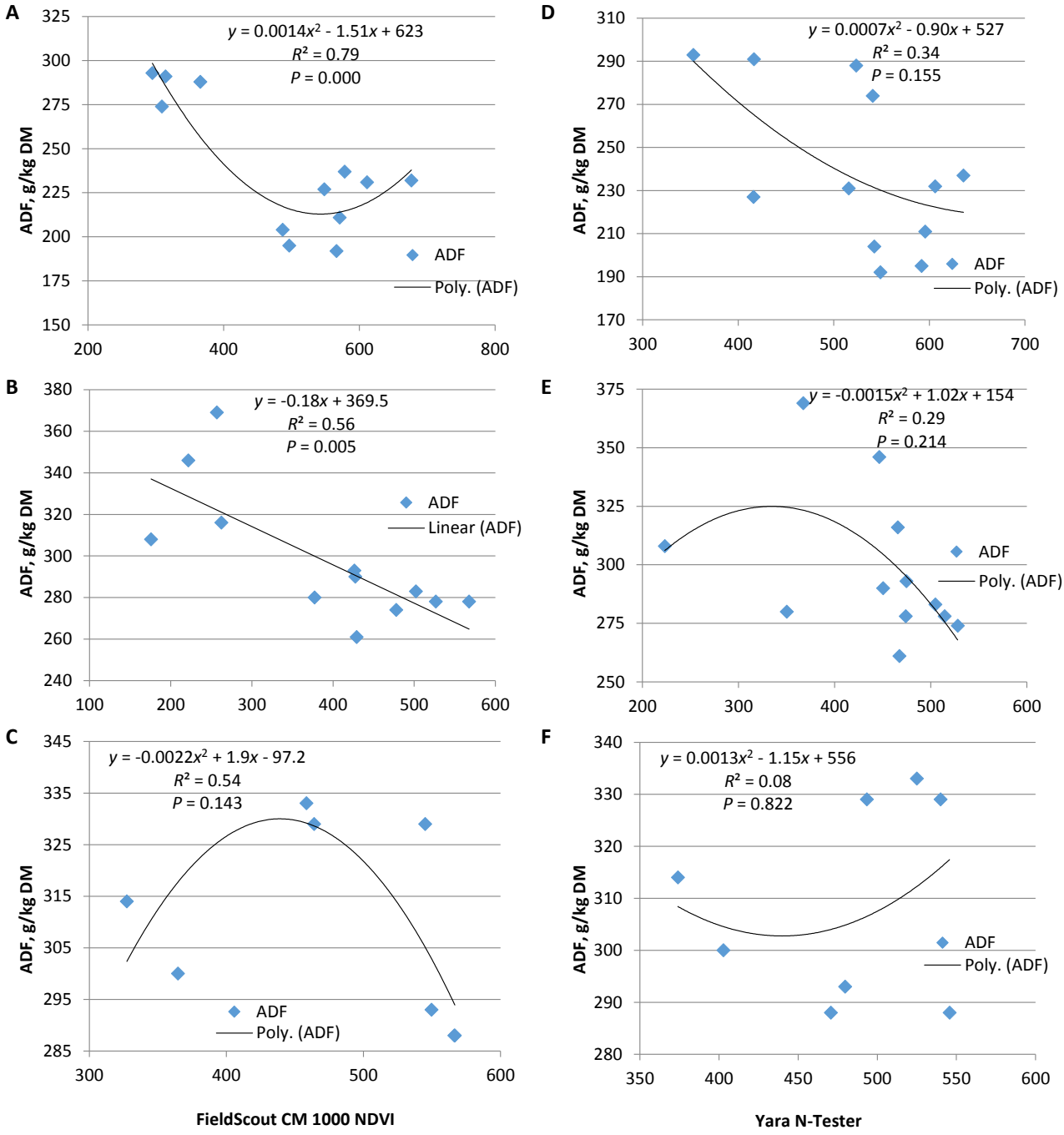

Fig. 2. Relationships between optical chlorophyll measurements and acid detergent fibre (ADF, g/kg DM) concentrations of *Brachiaria* hybrid (A and D), *Megathyrsus maximus* (B and E) and *Paspalum atratum* (C and F).

could negatively impact optical chlorophyll measurements (Monje and Bugbee, 1992). Moderate to high correlation coefficients between optical chlorophyll measurements, particularly with *Brachiaria* hybrid and *M. maximus* NDF and ADF from the FieldScout CM 1,000 NDVI, are similar to the report of Hughes et al. (2014) but inconsistent with the report of Starks et al. (2006). Hughes et al. (2014) reported *r* values (−0.71 to −0.72) between FieldScout CM 1,000 and ADF concentration of *B. decumbens* cv. Basilik pastures harvested at 14 and 13 days of regrowth post grazing. On the other hand, Starks et al. (2006) reported lower *r* values of −0.45 and −0.38 for Bermuda grass NDF and ADF concentrations, respectively from the portable FieldSpec NDVI reflectance measurements. Differences in these reports as well as differences between species in the present study may be attributed to a combination of factors such as variations in leaf morphology and canopy cover, which are related to the degree of light interception, exposed soil surface (Albayrak, 2008) and variations in species biochemical composition (Monje and Bugbee, 1992; Stark et al., 2006) possibly caused by stage of growth and proportion of leaf to stem which affects transmission of light through the leaf. Generally, *r* values for ADL were within the range of the previous report (0.02–0.72) by Hughes et al. (2014).

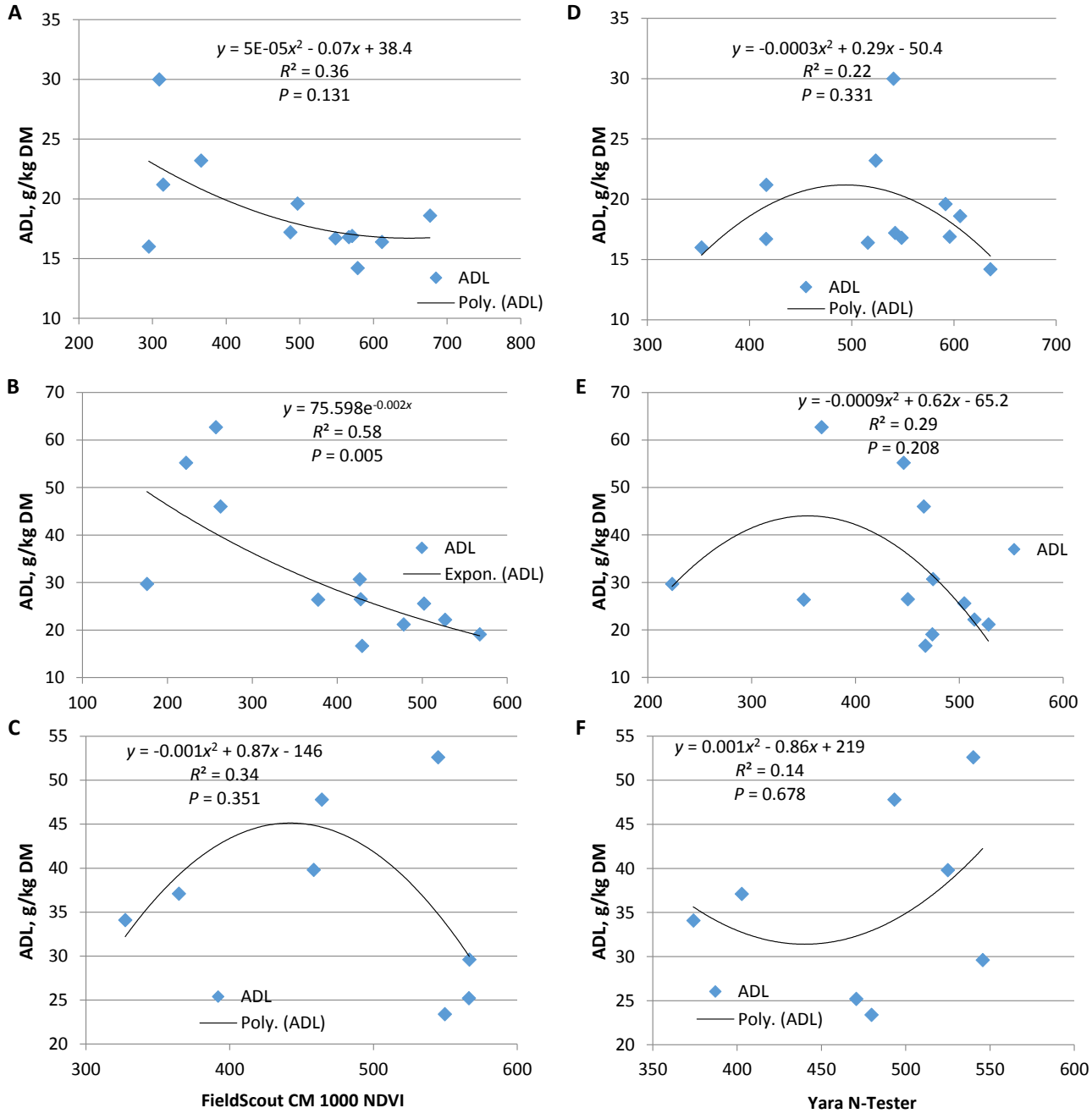

Fig. 3. Relationships between optical chlorophyll measurements and acid detergent lignin (ADL, g/kg DM) concentrations of *Brachiaria* hybrid (A and D), *Megathyrsus maximus* (B and E) and *Paspalum atratum* (C and F).

Starks et al. (2006) highlighted the fact that there are only a few reports relating to optical properties of pasture herbage nutritional characteristics such as fibre components. In fact, Hughes et al. (2014) is the only report found to date documenting relationships between optical chlorophyll measurements and lignin concentrations and ruminal degradability of tropical grass herbage. These authors found that the FieldScout CM 1,000 NDVI produced poor and unreliable estimates of lignin concentration in *B. decumbens* ($R^2 = 0.16 - 0.66$) but better predicted $IVOMD_{48h}$ ($R^2 = 0.50–0.78$). The relationship between foliar optical chlorophyll measurements and macro-constituents seems to be influenced by the relative proportions of each component within the cell-wall structure. These components are not uniform and dependent on growth state, environmental conditions and species. Additionally, Starks et al. (2006) suggested that canopy reflectance is influenced by a number of factors including vegetative ground cover, canopy architecture and biochemical composition of the plant tissue. In this study, NDF, which represents the largest fibre fraction, consistently returned the highest prediction power followed by ADF and then ADL. Similarly, Starks et al. (2006) observed a similar trend where canopy reflectance measurements in Bermuda grass pastures accounted for 23% and 21% of NDF and ADF variability, respectively. Coefficient of determination for NDF ($R^2 = 0.61 - 0.77$) and ADF ($R^2 = 0.68 - 0.75$) reported by Albayrak (2008) in the cool-season legume sainfoin *(O. sativa)* from a portable spectroradiometer (Analytical Spectral Devices Inc.; Boulder, CO, USA) were within the range of those from the

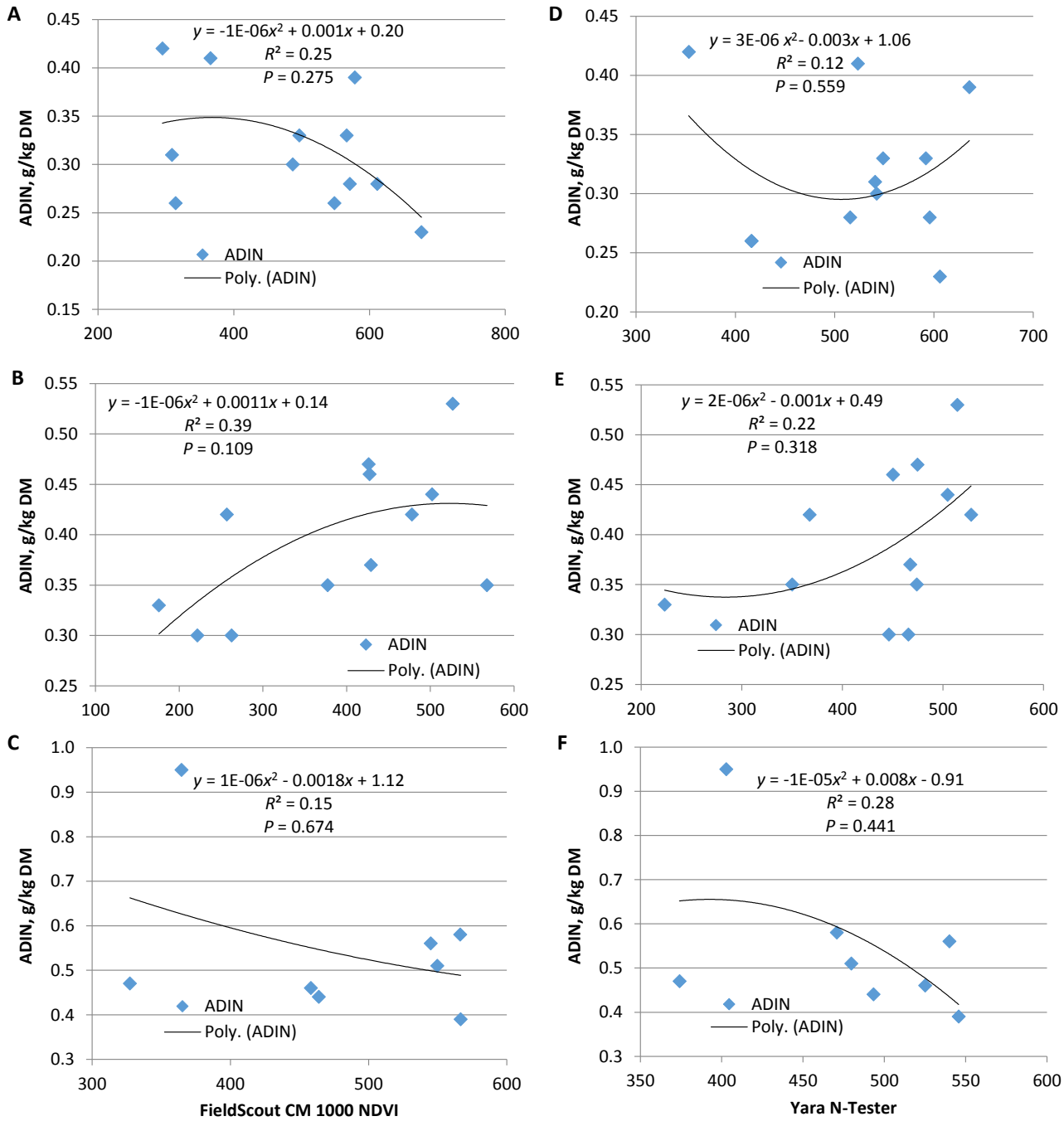

Fig. 4. Relationships between optical chlorophyll measurements and acid detergent insoluble nitrogen (ADIN, g/kg DM) concentrations of *Brachiaria* hybrid (A and D), *Megathyrsus maximus* (B and E) and *Paspalum atratum* (C and F).

present study. FieldScout CM 1,000 NDVI on all occasions produced higher prediction power for NDF, ADF and ADL compared with the Yara N-Tester. This suggests that the FieldScout CM 1,000 NDVI might be more sensitive to tissue chemical constituents and spectral reflectance than the Yara N-Tester.

Further, despite both devices measuring light absorbance at similar wavelengths, the FieldScout CM 1,000 NDVI measurements are based on grass canopy while the Yara N-Tester measurements are specific to the leaves, hence inclusion of stem material might negatively affect Yara N-Tester prediction power. Both FieldScout CM 1,000 NDVI and Yara N-Tester poorly predicted ADIN concentrations. No previous reports have sought to establish the relationship between optical chlorophyll measurements and pasture ADIN concentration. The inability of both optical chlorophyll devices to produce prediction models with high predictive power for ADIN concentration could be because of fibre-bound N, which forms the bulk of ADIN, is not a component of the chlorophyll molecule. Also, other cell wall components such as lignin could form a barrier between cell wall N and light transmittance by both devices.

Table 4
Relationship between (*Y*) *in vitro* organic matter degradability (IVOMD) and (*x*) optical chlorophyll measurements.

Species	IVOMD (Y) Regression model		R^2	*P*-value
FieldScout CM 1,000 NDVI				
Brachiaria hybrid	12 h	$Y = -0.002x^2 + 2.4x - 133$	0.62	0.013
	24 h	$Y = -0.0044x^2 + 4.4x - 373$	0.81	0.000
	48 h	$Y = -0.0022x^2 + 2.6x + 84.1$	0.65	0.008
Megathyrsus maximus	12 h	$Y = -0.0008x^2 + 0.89x + 167$	0.47	0.058
	24 h	$Y = 0.0002x^2 + 0.13x + 476$	0.32	0.181
	48 h	$Y = 0.0016x^2 - 0.57x + 643$	0.75	0.001
Paspalum atratum	12 h	$Y = 0.0023x^2 - 2.3x + 987$	0.35	0.346
	24 h	$Y = 0.0008x^2 - 1.01x + 868$	0.29	0.427
	48 h	$Y = 0.0048x^2 - 4.51x + 1,784$	0.22	0.540
Yara N-Tester				
B. hybrid	12 h	$Y = -0.0031x^2 + 3.7x - 583$	0.52	0.036
	24 h	$Y = -0.0029x^2 + 3.2x - 209$	0.23	0.311
	48 h	$Y = 0.0006x^2 - 0.08x + 653$	0.24	0.298
M. maximus	12 h	$Y = 203.48e^{0.0014x}$	0.53	0.036
	24 h	$Y = 0.0029x^2 - 1.76x + 748$	0.53	0.032
	48 h	$Y = 0.0043x^2 - 2.52x + 931$	0.65	0.002
P. atratum	12 h	$Y = 0.0035x^2 - 3.6x + 1,333$	0.32	0.381
	24 h	$Y = 0.008x^2 - 7.6x + 2,360$	0.32	0.386
	48 h	$Y = -0.0081x^2 + 6.77x - 597$	0.55	0.138

IVOMD = *in vitro* organic matter degradability (g/kg) post 12, 24 & 48 h incubation.

4.2. Relationships between optical chlorophyll measurements and IVOMD

From the only report describing relationships between optical chlorophyll measurements and grass forage IVOMD, Hughes et al. (2014) reported that the FieldScout CM 1,000 NDVI measurements accounted for 50%–78% variance of *B. decumbens* $IVOMD_{48h}$ dependent on pasture regrowth maturity, and was, therefore, capable of producing accurate and reliable estimates of $IVOMD_{48h}$. In the present study, the FieldScout CM 1,000 NDVI accounted for 81% and 75% of *Brachiaria* hybrid and *M. maximus* $IVOMD_{48h}$ variability, respectively, while the Yara N-Tester poorly estimated IVOMD in both species. The fact that chlorophyll and N are major components of the soluble cell fraction and N, in particular, is critical in mediating ruminal microbial activity sufficiently justifies this positive relationship.

Indeed, the high positive correlations between FieldScout CM 1,000 NDVI measurements and *Brachiaria* hybrid and *M. maximus* $IVOMD_{48h}$ and Yara N-Tester IVOMD could be an indication that these devices are more sensitive to macro-constituents of the leaf tissue such as fibre, lignin and CP (Jung and Allen, 1995; Hughes et al., 2014) that are known to influence forage degradability, particularly after 48 h incubation (Njidda and Ikhimioya, 2010). The optical measurement/IVOMD relationship was best described by polynomial models suggesting that factors other than N or chlorophyll significantly influenced IVOMD predictions. Therefore, chemical factors such as concentrations of fibre and lignin must be considered when making these predictions. The overall poor predictive power associated with $IVOMD_{12h}$ compared with $IVOMD_{48h}$ was surprising because chlorophyll and N occupy a large portion of the immediately soluble cell fraction that should be easily detected by the optical chlorophyll meters. Correlation analysis in the present study generally showed highest *r* values between optical chlorophyll measurements and $IVOMD_{48h}$. This could be an indication that CP and other immediately soluble cell constituents have their greatest influence on ruminal organic matter degradability within the initial stages of incubation. Indeed, Crawford et al. (1978) and Hvelplund and Weisbjerg (2000) suggested that for most feedstuffs, ammonia concentration usually peaks post 2 h ruminal exposure and the majority of feedstuff CP is degraded after just 3 h ruminal incubation, respectively. Since both meters operate within similar spectral bands, the better IVOMD prediction power from the

Table 5
Evaluation of selected prediction models: Relationships between optical chlorophyll measurements, chemical composition and *in vitro* ruminal organic matter degradability (IVOMD).

Item	*Brachiaria* hybrid						*Megathyrsus maximus*							*Paspalum atratum*					
	NDF	*NDF	ADF	IVOMD 48 h	IVOMD 24 h	*IVOMD 12 h	NDF	*NDF	ADF	ADL	IVOMD 48 h	*IVOMD 48 h	*IVOMD 24 h	IVOMD 12 h	*IVOMD 12 h	NDF	*NDF	ADF	*IVOMD 48 h
Mean																			
Observed	537	537	240	780	652	506	627	627	298	31.8	684	684	560	388	388	634	634	309	757
Predicted	555	565	242	793	656	529	621	625	300	35.9	687	683	580	380	379	633	637	291	760
CCC (0–1)	0.73	0.71	0.88	0.79	0.89	0.74	0.87	0.83	0.32	0.62	0.86	0.79	0.08	0.61	0.64	0.83	0.72	0.44	0.71
ρ (0–1)	0.83	0.82	0.89	0.81	0.90	0.78	0.88	0.85	0.54	0.72	0.87	0.81	0.65	0.68	0.71	0.85	0.79	0.72	0.74
C_b (0–1)	0.88	0.87	0.99	0.97	0.93	0.95	0.98	0.99	0.59	0.86	0.99	0.98	0.13	0.88	0.90	0.97	0.90	0.60	0.95
MSPE, g/kg	1,186	2,298	275	2,879	1,030	3,216	3,782	275	288	416	114	1,902	2,686	2,178	2,101	1,964	21.5	40.0	498	1,867
Partition of MSPE, %																			
Mean bias	28.3	33.9	2.27	5.56	1.81	16.6	13.5	15.7	1.39	0.74	14.7	0.36	0.12	19.2	3.18	4.35	6.13	32.1	37.3	
Regression bias	35.6	0.56	24.6	27.0	16.8	20.9	46.5	5.27	25.5	46.2	50.1	21.8	35.8	32.9	59.1	54.9	28.8	16.7	40.7	
Random error	36.1	65.5	73.2	67.4	81.3	62.5	40.0	80.0	73.1	53.1	35.3	77.8	64.1	47.9	37.7	40.7	65.0	51.3	22.1	

NDF = neutral detergent fibre inclusive of residual ash; ADF = acid detergent fibre; ADL = acid detergent lignin; $IVOMD_{12,\ 24\ \&\ 48\ h}$ = *in vitro* organic matter digestibility post 12, 24 and 48 h incubation; CCC = concordance correlation coefficient; ρ = correlation coefficient estimate, C_b = bias correction factor; MSPE = mean square prediction error.
*Denotes prediction models associated with the Yara N-Tester.

FieldScout CM 1,000 NDVI may be as a result of the FieldScout CM 1,000 NDVI being less affected by physical grass characteristics such as leaf and vein thickness. The FieldScout CM 1,000 NDVI would, therefore, be better able to account for chemical constituents of the whole plant and canopy compared with the Yara N-Tester measurements of only leaf, which have significant effects on IVOMD.

The advent of portable NIRS machines offers competition towards development of optical chlorophyll meters. Portable NIRS spectral data represents direct measures of a larger number of proximate components (fibre, lignin, protein, and other organic components) than chlorophyll meters that only measure chlorophyll concentration. Therefore, NIRS prediction power should be much better. However, compared with portable NIRS, optical chlorophyll meters are more affordable, particularly for resource-poor countries, more farmer friendly because they are easy to operate – requiring little technical skills and facilities and not entirely dependent on time and resource consuming calibration exercise.

4.3. Model evaluation

FieldScout CM 1,000 NDVI prediction models for *Brachiaria* hybrid ADF and $IVOMD_{24h}$ and *M. maximus* NDF and $IVOMD_{48h}$ were the best prediction models. FieldScout CM 1,000 NDVI measurements accounted for $\geq$75% of the variation in these variables. Unexplained portions of these variables can be accounted for by variations in leaf thickness, biochemical distribution and moisture content (Chang and Robison, 2003) brought about by different stages of grass maturity. An examination of the CCC revealed that these models fall marginally short of the acceptable level. Indeed, McBride (2005) suggested that the models with CCC less than 0.90 are considered poor. The CCC, otherwise called reproducibility index, simultaneously measures model accuracy and precision. Therefore, with high ($\geq$0.87) bias correction factor – C_b, which tests model accuracy and/or correlation coefficient estimate – ρ, a measure of model precision, these models can be useful. Further calibrations with larger data set are, therefore, recommended.

Mean square prediction error is probably the most widely used and reliable measure of goodness-of-fit for mathematical models (Tedeschi, 2006). However, MSPE is negatively affected by small sample size. Despite this, the relatively low MSPE in the present study and the fact that the major source of error associated with these models was random error, further validate the quality of the predictor and, therefore, suggest that these models are of acceptable accuracy. Chang and Robison (2003) recommended that statistically significant prediction models from optical chlorophyll measurements with acceptable R^2 and low variation between observed and predicted values may be useful for comparative purposes where relative and not absolute estimates are required. For models with low R^2 and CCC, unacceptability is further confirmed where the majority or a large proportion of their errors are mean or regression bias.

5. Conclusion

Both the FieldScout CM 1,000 NDVI and Yara N-Tester produced poor and unreliable estimates of ADIN and ADL concentrations in all 3 species. However, the FieldScout CM 1,000 NDVI showed greater potential than the Yara N-Tester to produce accurate estimates of fibre and OM degradability particularly $IVOMD_{24h}$ and ADF in *Brachiaria* hybrid and $IVOMD_{48h}$ and NDF concentrations in *M. maximus*.

Conflict of interest declaration

The authors declare there are no actual or potential conflicts of interest associated with this work.

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