

# Predicting Pre-harvest Forage Nutritive Value of Spring and Summer Growth of Alfalfa–Grass Mixtures

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## ABSTRACT

Currently available regression equations developed to predict pre-harvest nutritive attributes from simple field measurements taken in alfalfa (*Medicago sativa* L.)–grass mixtures can help producers determine when to best harvest their forages but they are applicable to only spring growth. Our objective was to develop and validate predictive equations of pre-harvest forage nutritive attributes of mixed alfalfa–grass stands for the spring and first summer growth cycles. Forage samples ( $n = 1856$ ) were collected in 2015 and 2016 from three research sites in Quebec, Canada, and used to develop predictive equations that were then validated using samples ( $n = 315$ ) collected on commercial farms across Quebec and compared to equations previously developed in New York State for use during spring growth. For newly developed equations with two to four field measurements,  $R^2$  ranged from 0.70 to 0.84. The best equation developed to estimate a neutral detergent fiber assayed with a heat stable  $\alpha$ -amylase and corrected for the ash content of the residue (aNDFom) had an  $R^2$  of 0.82 and a root mean square error (RMSE) of 29.3 g kg<sup>-1</sup> dry matter (DM). Some equations can be used to predict aNDFom concentration and the relative feed value of samples from commercial farms, but only if alfalfa proportion can be precisely determined. Locally developed equations resulted in better predictions than equations developed only for spring growth in New York State. Forage producers now have access to a tool to predict the pre-harvest nutritive value of their forages for two growth cycles.

## Core Ideas

- Predictive equations can help producers determine when to harvest their forage fields.
- Equations developed can predict nutritive value of alfalfa–grass stands for the spring and first summer growth cycles.
- Alfalfa proportion must be precisely determined for equations to yield reliable results.

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**T**HE NUTRITIVE value of forage crops is greatly affected by the timing of the harvests, which in turn influences ruminant animal production. In dairy production, feeding forages with an optimal nutritive value can contribute to maximize milk production from forages and thus reduce requirements for concentrated feeds, which in turn may increase farm profitability (Mertens, 2009; Pellerin et al., 2013). Forage nutritive value is determined based on several key attributes, which includes digestibility, and fiber and protein concentrations. However, forage nutritive attributes are often only assessed at the time of feeding and are less frequently a consideration in the field when deciding when to harvest. Indeed, many forage producers often base their harvest decisions on the calendar day, yield, or may rely on visual indicators such as plant developmental stages. This is attributable to a general lack of reliable, rapid, simple, and affordable methods to estimate forage nutritive attributes in the field prior to harvest. If multiple attributes can be used, the neutral detergent fiber (NDF) concentration is one attribute

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**Abbreviations:** ADFom, acid detergent fiber corrected for the ash content of the fiber residue; AFRAC, alfalfa fraction; AMAXHT, length in centimeters of the tallest alfalfa stem from the ground to the terminal bud once fully extended; aNDFom, neutral detergent fiber assayed with a heat-stable  $\alpha$ -amylase and corrected for the ash content of the fiber residue; ASTAGE, stage of development of the most mature alfalfa stem; CP, crude protein;  $d$ , index of agreement; DM, dry matter; DOY, day of year; GMAXHT, length in centimeters of the tallest grass stem from the ground to the tip of the lastly emerged grass leaf; GDD, cumulated growing degree days; GFRAC, grass fraction; GGRP, grass fraction group; GSTAGE, stage of development of the most mature grass tiller; IVTDom, in vitro true digestibility of dry matter corrected for the ash content of the residue; NDF, neutral detergent fiber; NDFdom, in vitro neutral detergent fiber digestibility corrected for the ash content of the residue; NYPEAQ, predictive equations of forage nutritive attributes from New York State; RFV, relative feed value; RMSE, root mean square error; TN, total nitrogen; VNIRS, visible near-infrared reflectance spectroscopy.

often used to determine the timing of harvests. Recommended target NDF concentrations at the time of harvest vary depending on the forage species and the animal to be fed, but have been reported to range between 400 and 550 g kg<sup>-1</sup> DM (Cherney et al., 2006; Parsons et al., 2009; Undersander et al., 2014); deviations from these values potentially may have substantial impacts on animal productivity and farm profitability.

Several methods based on weather or plant development have been developed to estimate forage nutritive attributes in the field prior to harvesting (Fick and Onstad, 1988; Fick et al., 1994; Allen and Beck, 1996). The predictive equations of alfalfa quality (PEAQ) were originally developed in Wisconsin and use simple field measurements, such as plant height and stage of maturity, to predict the pre-harvest NDF and acid detergent fiber (ADF) concentrations of pure alfalfa stands (Hintz and Albrecht, 1991). The PEAQ model was demonstrated to provide robust predictions of alfalfa fiber concentrations in a range of environments in North America and Europe (Sulc et al., 1997; Santillano-Cázares et al., 2014; Hakl et al., 2010; Andrzejewska et al., 2014). It has also been adapted for use with alfalfa–grass mixtures that predominate in many regions of northeastern North America, including the Province of Quebec, Canada. Parsons et al. (2006a, 2013) developed predictive equations of nutritive attributes of the spring growth of alfalfa–grass mixtures for New York State. These equations (NYPEAQ) use field measured variables including the grass contribution to total yield (grass fraction [GFRAC]), maximum alfalfa plant height, and growing degree day (GDD) accumulation to predict important nutritive attributes, including NDF and ADF concentrations, relative feed value (RFV), in vitro neutral detergent fiber digestibility (NDFd), and relative forage quality (RFQ). A validation study recently conducted in the Province of Quebec demonstrated that NYPEAQ equations could be used outside of their area of development to predict both NDF concentration and RFV (Wood et al., 2018). These predictive equations were validated using samples and data collected by trained personnel at experimental research sites and the important variable GFRAC was determined precisely using hand separated samples. However, if agronomists or agricultural producers are to use predictive equations, it is essential that validation be confirmed using data collected by a wide range of individuals with some variables such as GFRAC possibly being visually estimated to simplify data collection. In addition, to maximize the use and impact of predictive equations, it is also essential to develop equations for multiple growth cycles, as current NYPEAQ equations were developed to only predict nutritive attributes of the spring growth.

The objective of the current study was thus to develop and validate predictive equations of the pre-harvest forage nutritive attributes of mixed alfalfa–grass stands for multiple growth cycles. Predictive equations were developed using data from three contrasted experimental research sites in the Province of Quebec, Canada, and then validated using samples collected on commercial farms across 12 of the 17 administrative regions of the Province.

## MATERIALS AND METHODS

### Field Description

For the development of predictive equations of forage attributes (Part 1), experimental plots were established at three climatically-contrasted sites in the Province of Quebec, Canada.

Alfalfa–grass mixtures were seeded in May 2014 at Sainte-Anne-de-Bellevue, QC (45°43' N, 73°94' W), Lévis, QC (46°80' N, 71°09' W), and Normandin, QC (48°50' N, 72°32' W). At each site, two experimental plot series were sown with one being sampled in 2015 and the other in 2016. Wood et al. (2018) previously reported soil characteristics at each site. Mixtures of alfalfa (cultivar Calypso) with timothy (*Phleum pratense* L. 'AC Alliance') or tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., formerly *Festuca arundinacea* Schreb. 'Courtenay']] were seeded in the following proportions: 80:20, 60:40, 40:60, and 20:80 resulting in eight treatments. Wood et al. (2018) previously described in detail seeding and plot management procedures. Briefly, plots of the eight alfalfa and grass proportion treatments were seeded at the following rates (based on weight of pure live seeds): 12.8 and 3.2, 9.6 and 6.4, 6.4 and 9.6, and 3.2 and 12.8 kg ha<sup>-1</sup>, for alfalfa and timothy, and 15.2 and 3.8, 11.4 and 7.6, 7.6 and 11.4, and 3.8 and 15.2 kg ha<sup>-1</sup>, for alfalfa and tall fescue, respectively. Treatments were duplicated at each site with one plot being sampled during spring growth and the second during the first summer regrowth. Plots were randomly assigned to a randomized complete block design with a split-plot restriction and two replications. Main plots were assigned to either spring growth or first summer regrowth, while subplots were assigned to the alfalfa–grass proportion treatments. Each subplot measured a minimum of 1.3 by 5 m; the exact size depending on the site.

For the validation of the predictive equations (Part 2), samples and data were collected from fields on commercial farms across the Province of Quebec. Samples were collected from fields located in 12 of the 17 administrative regions of Quebec (i.e., Abitibi-Témiscamingue, Bas-Saint-Laurent, Capitale-Nationale, Centre-du-Québec, Chaudière-Appalaches, Estrie, Gaspésie-Îles-de-la-Madeleine, Laurentides, Montérégie-Ouest, Lanaudière, Mauricie, and Outaouais). Ten fields were sampled in 2015 and 13 in 2016. Selected fields were predominantly mixtures of alfalfa with either timothy or tall fescue. Fields varied in terms of soil characteristics, seeding, fertilization, and harvest management. Two areas of 8 m<sup>2</sup> were randomly selected in each field for data collection and sampling.

### Field Sampling and Measurements

For Part 1 of the study, a total of 1856 samples were collected from experimental plots (908 in 2015 and 908 in 2016). Each plot was sampled twice a week for 4 or 5 wk during both the spring growth and the first summer regrowth using 50 by 50 cm quadrats, with each sample being taken from a previously unsampled area. Sampling in the spring was initiated when alfalfa reached an average height of 40 cm and continued for up to 5 wk resulting in 8 to 10 independent samples per plot. Plots sampled during the spring growth were then discarded. Plots to be sampled during the first summer regrowth were all cut on the same date when the spring growth of alfalfa reached the early flowering stage of development. These plots were then allowed to regrow to an average alfalfa height of 30 cm before being sampled during the first summer regrowth using the same method as described previously for the spring growth sampling.

For Part 2 of the study, a total of 315 samples (144 in 2015 and 171 in 2016) were collected from commercial farm fields. Plots were sampled twice a week for up to 4 wk during the spring growth once the alfalfa reached an average height of 40 cm.

Samples were taken within a 50 by 50 cm quadrat with each sample being taken from a previously unsampled portion from the 8-m<sup>2</sup> area, resulting in up to eight independent samples per field.

Measurements and staging of plants within the quadrats for Part 1 of the study were completed as Wood et al. (2018) described in detail and included: alfalfa maximum height (AMAXHT, length in centimeters of the tallest alfalfa stem from the ground to the terminal bud once fully extended); alfalfa maximum stage (ASTAGE, stage of development of the most mature alfalfa stem) based on Kalu and Fick (1981); grass maximum height (GMAXHT, length in centimeters of the tallest grass stem from the ground to the tip of the lastly emerged grass leaf); and grass maximum stage (GSTAGE, stage of development of the most mature grass tiller) based on Moore and Moser (1995). Samples were cut at a height of 7.5 cm using scissors and were later separated into alfalfa, grass, and weed components before being bagged and dried at 55°C for 72 h to determine the actual grass and alfalfa contributions to total biomass on a DM basis (GFRAC and alfalfa fraction [AFRAC]). Forage DM yield per hectare was also determined from these samples. The grass fraction group (GGRP) was defined as the 0.20 interval (i.e., 0.2, 0.4, 0.6, or 0.8) that was closest to the GFRAC value. The day of the year (DOY) that is the number of days from the start of the year at time of sampling was noted and data for the calculation of cumulated growing degree days (GDD) were collected from weather stations located at each site. Growing degree days in degrees Celsius using a base temperature of 0°C (GDD0) and 5°C (GDD5) were calculated as Wood et al. (2018) described. The data collected and mean, range, and standard deviation for each variable determined are presented in Supplemental Table S1.

For Part 2 of the study, the data collection on commercial farm fields was similar to that described above and included: AMAXHT, ASTAGE, GMAXHT, GGRP, GSTAGE, DOY, GDD0, and GDD5. The determination of alfalfa and grass contributions to total biomass, however, differed. The GFRAC was estimated visually, while the AFRAC was determined by subtracting GFRAC from 1 assuming that samples were weed-free. Samples were cut at a height of 7.5 cm using scissors, bagged, and placed in freezers until all samples were collected. All samples were then dried at 55°C for 72 h. Data for the calculation of GDDs were collected from the nearest weather station that could be accessed through the Environment Canada meteorological website (<http://climate.weather.gc.ca/>). The data collected and mean, range, and standard deviation for each determined variate are presented in Supplemental Table S2.

### Nutritive Value Analyses

Wood et al. (2018) described in detail sample preparation, details of laboratory analyses, and appropriate calculations. Briefly, all collected forage samples from both parts of the study were scanned by visible near infrared reflectance spectroscopy (VNIRS) using a NIRS DS2500 monochromator instrument (Foss NIRSystems Inc., Silver Spring, MD). From the VNIRS scans of the samples taken for Part 1 of the study, a set of 170 forage samples (120 for calibration, 30 for validation, and 20 outliers) was selected by the WinISI software version 4.5.0.1407 (Infrasoft International, LLC, Silver Spring, MD). Using the same procedure, a validation set of 43 forage samples (22 from 2015 and 21 from 2016) was selected from samples collected

from commercial farms for Part 2 of the study. The sets of 170 and 43 forage samples were chemically analyzed for the concentrations of acid detergent fiber corrected for the ash content of the fiber residue (ADFom), aNDFom, total nitrogen (TN) to be converted to crude protein (CP = TN × 6.25), in vitro true digestibility of dry matter corrected for the ash content of the residue (IVTDom), and in vitro neutral detergent fiber digestibility corrected for the ash content of the residue (NDFdom). The relative feed value (RFV) was calculated using the Excel spreadsheet Milk2013 (Undersander et al., 2013) for all chemically analyzed forage samples. Results from chemical analyses and calculations for the calibration set of samples were then used to develop calibration equations for each nutritive attribute using the WinISI 4 software. Calibration equations were validated using the same software by comparing predicted against reference laboratory values obtained for the 30 validation samples selected for the development of predictive equations. The VNIRS predictions were considered excellent with the ratio of prediction to deviation (RPD = ratio of standard deviation of the reference data used in the validation set to standard error of prediction corrected for bias) values >4, ranging between 4.4 and 5.4 as Wood et al. (2018) reported. Using the modified PLSR method of the WinISI 4 software, predicted values of each nutritive attribute were then generated for all forage samples of the development and validation of the predictive equations. For samples collected on commercial farms in Part 2 of the study, the VNIRS predictions were considered successful with RPD values >3 for ADFom, aNDFom, and CP, moderately successful for NDFdom with 2.25 < RPD < 3, and moderately useful for IVTDom and RFV with 1.75 < RPD < 2.25.

Given that the AFRAC and GFRAC values for samples of Part 2 of the study were visually estimated by a large number of participants, we also estimated the AFRAC values of these samples with a VNIRS-predictive equation developed using a similar procedure as described above. Using the manually determined AFRAC values in all forage samples from Part 1 of the current study and from three previous experiments, a robust VNIRS-equation was developed to estimate this attribute in mixed alfalfa–grass samples. The VNIRS predictions obtained using this procedure were considered successful with an RPD of 3.52. This VNIRS equation was used to estimate the AFRAC of all samples from commercial farms. The GFRAC was also determined by subtracting AFRAC from 1 assuming that samples were weed free. These estimated values of AFRAC and GFRAC will later be referred to as AFRAC and GFRAC determined by VNIRS. The mean, range, and standard deviation for the nutritive attributes of samples from commercial farms are presented in Supplemental Table S2.

### Data Analysis

Data from experimental plots of Part 1 of the study were used for the development of predictive equations for the nutritive attributes of both the spring growth and the first summer regrowth of alfalfa–grass mixtures. To ensure that only samples with both alfalfa and grasses were used, all samples with less than 10% or more than 90% grass (based on GFRAC values) were removed from the set of 1856, leaving a total of 1156 samples for equation development. The data were then analyzed using PROC REG in SAS (SAS version 9.4, SAS Institute,

Table 1. Equations developed using data of alfalfa–grass mixture samples from the spring growth and the first summer regrowth at three sites in Quebec, Canada, to predict neutral detergent fiber (aNDFom; g kg<sup>-1</sup> dry matter [DM]), acid detergent fiber (ADFom; g kg<sup>-1</sup> DM), in vitro neutral detergent fiber digestibility based on a 48-h incubation (NDFdom; g kg<sup>-1</sup> aNDF), in vitro true digestibility based on a 48-h incubation (IVTDom; g kg<sup>-1</sup> DM), all corrected for the organic matter content of the residues, crude protein (CP) (g kg<sup>-1</sup> DM), and relative feed value (RFV) (*n* = 1156).

Equation	R <sup>2</sup> †	RMSE	NRMSE	Mean 95% CI
Eq. [1]: aNDFom = 384 + (2.60 × AMAXHT) – (230 × AFRAC)	0.78	32.0	10.1	63.0
Eq. [2]: aNDFom = 356 + (1.79 × AMAXHT) + (0.93 × GMAXHT) – (203 × AFRAC)	0.81	30.1	9.6	59.1
Eq. [3]: aNDFom = 352 + (6.88 × ASTAGE) + (1.34 × AMAXHT) + (1.05 × GMAXHT) – (205 × AFRAC)	0.82	29.3	9.3	57.6
Eq. [4]: ADFom = 159 + (1.29 × AMAXHT) + (0.83 × GMAXHT)	0.77	21.2	11.2	41.7
Eq. [5]: ADFom = 155 + (5.42 × ASTAGE) + (0.93 × AMAXHT) + (0.93 × GMAXHT)	0.79	20.5	10.8	40.3
Eq. [6]: ADFom = 144 + (0.90 × AMAXHT) + (0.77 × GMAXHT) + (22.6 × GGRP) + (0.10 × GDD5)	0.81	19.7	10.4	38.7
Eq. [7]: NDFdom = 1069 – (0.37 × GDD0) – (276 × AFRAC)	0.80	40.6	9.2	79.7
Eq. [8]: NDFdom = 829 – (1.64 × AMAXHT) – (0.22 × GDD0) + (250 × GFRAC)	0.83	37.1	7.9	73.0
Eq. [9]: NDFdom = 934 – (1.55 × AMAXHT) – (0.23 × GDD0) + (142 × GFRAC) – (112 × AFRAC)	0.84	36.6	7.9	72.0
Eq. [10]: IVTDom = 1018 – (7.15 × ASTAGE) – (1.96 × AMAXHT)	0.77	25.2	10.6	49.4
Eq. [11]: IVTDom = 1012 – (1.36 × AMAXHT) – (0.13 × GDD0) + (42.3 × GFRAC)	0.80	23.2	9.6	45.6
Eq. [12]: IVTDom = 1016 – (0.90 × AMAXHT) – (0.73 × GMAXHT) + (41.5 × GGRP) – (0.16 × GDD5)	0.82	22.2	9.9	43.7
Eq. [13]: CP = 340 – (0.19 × GDD0) – (77.0 × GFRAC)	0.70	22.6	12.4	44.5
Eq. [14]: CP = 265 – (2.66 × ASTAGE) – (0.17 × GDD0) + (80.3 × AFRAC)	0.71	22.5	12.2	44.2
Eq. [15]: CP = 302 – (2.73 × ASTAGE) – (0.17 × GDD0) – (40.8 × GFRAC) + (42.4 × AFRAC)	0.71	22.4	12.0	44.0
Eq. [16]: RFV = 165 – (1.17 × AMAXHT) + (89.9 × AFRAC)	0.76	14.2	10.9	27.8
Eq. [17]: RFV = 185 – (0.67 × GMAXHT) – (0.10 × GDD5) + (66.5 × AFRAC)	0.79	13.4	9.8	26.3
Eq. [18]: RFV = 178 – (3.62 × ASTAGE) – (0.61 × AMAXHT) – (0.44 × GMAXHT) + (78.0 × AFRAC)	0.80	12.9	9.7	25.4

† R<sup>2</sup>, coefficient of determination; RMSE, root mean square error (the unit is the same as the nutritive attribute); NRMSE, normalized root mean square error (%); CI, confidence interval (CI; the unit is the same as the nutritive attribute). AFRAC, alfalfa fraction of sample written as a decimal; AMAXHT, length in centimeters of the tallest alfalfa stem from the ground to the terminal bud once fully extended; ASTAGE, stage of development of the most mature alfalfa stem alfalfa maximum stage based on Kalu and Fick (1981); DOY, day of year; GDD0, cumulated growing degree days based on 0°C; and GDD5, cumulated growing degree days based on 5°C; GFRAC, grass fraction of sample written as a decimal (e.g., 0.1 or 0.6); GGRP, grass fraction group written as a decimal (e.g., 0.2, 0.4, 0.6, and 0.8); GMAXHT, grass maximum height in cm of the tallest grass stem from the ground to the tip of the lastly emerged grass leaf.

Cary, NC) with predictive equations being identified using the RSQUARE method to select models that maximized the coefficient of determination (*R*<sup>2</sup>) with a targeted minimum of 0.75. The single best two-, three-, and four-variable predictive equations were selected for each forage nutritive attribute of interest based on their *R*<sup>2</sup>, as well as root mean square error (RMSE), normalized root mean square error (NRMSE; RMSE divided by the mean of observed values), and mean 95% confidence interval (CI), with minimal values being targeted for these later three criteria (Myers, 1990).

For the validation of these newly created predictive equations, statistical analysis of their results using data collected on commercial farms from Part 2 of the study was performed using PROC REG in SAS (SAS version 9.4, SAS Institute, Cary, NC). Data were entered into the predictive equations developed as described above. Only predictive equations that had an *R*<sup>2</sup> value ≥ 0.75 were validated. Wood et al. (2018) previously described details and procedures used for validation. Briefly, the predictive equations were validated by linearly regressing observed values on the corresponding values predicted using the various predictive equations developed in Part 1 of the study, as well as a few selected equations developed by Parsons et al. (2013) that had shown potential for use in Quebec (Wood et al., 2018). The mean observed and predicted values for each equation were reported, as were the *R*<sup>2</sup> of the simple linear regression between the observed and predicted values along with the RMSE. The slope (*b*) and intercept (*a*) of all regression lines were tested for the hypothesis that the slope was not significantly different from 1.0 and the intercept was not significantly different from 0. The index of agreement (*d*) was also calculated

to further define the relation between observed and predicted values (Willmott, 1982). This index reports on the degree of accuracy of the prediction and varies between 0 and 1.0, with 1.0 indicating a perfect agreement between values, while 0 indicates a complete disagreement.

## RESULTS AND DISCUSSION

### Part I: Development of Predictive Equations of Forage Nutritive Attributes for Multiple Growth Cycles of Mixed Alfalfa–Grass Stands

Many predictive equations were developed for each nutritive attribute but only the single best two-, three-, and four-variable equations were selected principally based on *R*<sup>2</sup> and RMSE values. Only predictive equations that met our a priori defined threshold of an *R*<sup>2</sup> ≥ 0.75 were selected and presented for all nutritive attributes except CP concentration, for which a maximum *R*<sup>2</sup> of 0.71 was obtained (Table 1). In general, increasing the number of variables included in the predictive equations led to greater *R*<sup>2</sup> values and lower RMSE for all nutritive attributes. The *R*<sup>2</sup> values of the best predictive equations ranged from 0.70 to 0.84, with values for aNDFom, ADFom, NDFdom, and RFV being similar to *R*<sup>2</sup> values that Parsons et al. (2013) reported (0.60 < *R*<sup>2</sup> < 0.81) for equations developed in New York State to predict nutritive attributes of the spring growth of mixed alfalfa–grass stands. In the specific case of aNDFom, the *R*<sup>2</sup> values in the current study ranged between 0.78 and 0.82 and were comparable to those that Parsons et al. (2013) reported (0.73 < *R*<sup>2</sup> < 0.81) but were lower than those of Parsons et al. (2006a) (0.89 < *R*<sup>2</sup> < 0.94). The RMSE for some attributes were often comparable to values previously reported in the development of predictive

equations for alfalfa or mixed alfalfa–grass stands in other regions (Hintz and Albrecht, 1991; Parsons et al., 2013).

To our knowledge, the current study is the first to report predictive equations for IVTDom with the resulting best two-, three-, and four-variable equations having  $R^2$  values as high as 0.77 to 0.82 (Table 1). None of the predictive equations for CP concentration met our minimum threshold of  $R^2 \geq 0.75$ , the highest value being 0.71. Such values are in accordance with previous attempts to develop predictive equations for CP concentration of pure alfalfa stands (Fick and Janson, 1990; Hintz and Albrecht, 1991).

The most prevalent variable in our equations was alfalfa maximum height in centimeters (AMAXHT), a variable previously reported to be important when predicting the nutritive value of pure alfalfa stands (Hintz and Albrecht, 1991; Parsons et al., 2006b; Andrzejewska et al., 2014) and alfalfa–grass mixtures (Parsons et al., 2006a, 2013). Other variables of importance, based on the number of appearances in our equations, relate to the botanical composition of the forage mixture, with these being either GFRAC (i.e., the grass contribution to forage DM yield) or AFRAC (i.e., the alfalfa contribution to forage DM yield). For all nutritive attributes, a botanical composition variable was included in all or the majority of the equations with the exception of ADFom and IVTDom. The grass fraction was also present in most equations proposed by Parsons et al. (2006a, 2013) for predicting the nutritive attributes of legume–grass mixtures in the spring growth. A growing degree day variable, either GDD0 (i.e., growing degree-days base 0°C) or GDD5 (i.e., growing degree-days based 5°C), was also included in 10 of our 18 best equations, especially the three- and four-variable equations, with the exception of those predicting aNDFom. Two other variables, GMAXHT and ASTAGE, were also included in some equations but mostly in the three- and four-variable equations.

Our results for these newly developed predictive equations are difficult to compare to those reported in the literature as, to our knowledge, this is the first attempt to develop equations to predict the nutritive attributes of mixed alfalfa–grass stands from both the spring and the first summer regrowth. Existing predictive equations were developed for either the spring growth of mixed alfalfa–grass stands (Parsons et al., 2006a, 2013) or multiple growth cycles of pure alfalfa stands (Hintz and Albrecht, 1991), but none combined the two concepts of mixed alfalfa–grass and multiple growth cycles. It was expected that the additional complexity resulting from the addition of the grass component would lead to equations with less predictive ability; this was confirmed by our results. Indeed, for example, our equations predicting the aNDFom concentration of alfalfa–grass mixtures resulted in lower  $R^2$  (0.78–0.82 vs. 0.92) and higher RMSE (29.3–32.0 vs. 22.1 g kg<sup>-1</sup> DM) than those Hintz and Albrecht (1991) reported for their equation predicting aNDF concentration for multiple growth cycles of pure alfalfa stands.

As mentioned earlier, our predictive equations for aNDFom concentration in the spring growth and the first summer regrowth had lower  $R^2$  and higher RMSE than those reported for aNDF concentration by Parsons et al. (2006a, 2013) for the spring growth. When using only our data from the spring growth for developing the predictive equations, the  $R^2$  and RSME values were closer to those Parsons et al. (2006a) reported (data not shown). This suggests that differences in regrowth patterns of different forage species after the first harvest may have affected predictions of their nutritive value. This is plausible as the two grass species in the current study differ in their development after the spring harvest with tall fescue

usually not producing reproductive structures during its regrowth (Wolf et al., 1979) contrary to timothy (Berg et al., 1996). It has also been shown that post-harvest regrowth of forages can mature faster at shorter canopy heights due to increased stressors such as higher temperatures and the potential decrease of water availability during the summer period, which can lead to dormancy in some cool-season grasses (Van Soest, 1985). This may have affected the relationship between the maximum height of alfalfa and the grasses, and the nutritive attributes during the first summer regrowth.

## Part 2: Validation of Predictive Equations on Commercial Farms

Some of the most promising predictive equations developed in Part 1 of the study were evaluated for their potential in predicting nutritive attributes of alfalfa–grass mixtures using data collected on commercial farms from 12 administrative regions of the Province of Quebec. The objective was to determine how well these predictive equations perform in non-research situations, using samples and data collected from the spring growth of alfalfa–grass mixtures taken by many participants who ranged in experience working with forages and in measuring the various variables required in those equations. In addition, some of the predictive equations that Parsons et al. (2013) developed only for the spring growth, and previously demonstrated to hold potential for use in Quebec according to data from experimental research sites (Wood et al., 2018), were also evaluated using the same dataset. At the onset of experimentation, we established that a coefficient of determination ( $R^2$ ) of the linear regression  $\geq 0.75$  between observed and predicted values would be the minimum acceptable to use the predictive equations across the Province of Quebec.

### Validation Using Visually Estimated Alfalfa and Grass Proportions

The predictive equations developed in the current study did not meet our minimum  $R^2$  criteria, with all of the equations resulting in  $R^2$  values of the linear regression between observed and predicted values lower than 0.75 (Table 2). The highest  $R^2$  values for each nutritive attribute ranged from 0.57 for NDFdom to 0.67 for aNDFom and RFV, while the lowest NRMSE for each nutritive attribute ranged from 4.1% for IVTDom to 12.8% for RFV. The slope of the linear regression between observed and predicted values was significantly different from the ideal value of 1 for all equations except those for ADFom. The intercept was significantly different from the ideal value of 0 for all aNDFom and NDFdom equations and two of three equations for RFV. All equations predicting ADFom and IVTDom and one equation predicting RFV, however, had intercepts that were not significantly different from the ideal. The validation statistics with the predictive equations developed in this study were better than those associated with the equations of Parsons et al. (2013) for the three nutritive attributes common to both studies, that is, aNDFom, ADFom, and RFV. Developing locally adapted equations therefore improved the predictions of nutritive attributes in the context, and with the conditions specific, to the present study.

A majority of the predictive equations included a botanical composition variable (AFRAC or GFRAC), which is difficult to visually estimate, as Parsons et al. (2006a, 2013) indicated and as supported by our own experience. The predictive equations

Table 2. Validation of equations developed in Quebec to predict nutritive attributes of mixed alfalfa–grass fields in the spring growth and the first summer regrowth and concurrent validation of comparable predictive equations developed for the spring growth in New York State. Values entered into equations for grass fraction (GFRAC) and alfalfa fraction (AFRAC) were visually estimated. Data were collected from commercial farm fields located across 12 administrative regions of Quebec. These equations predict the following nutritive attributes: neutral detergent fiber (aNDFom; g kg<sup>-1</sup> DM), acid detergent fiber (ADFom; g kg<sup>-1</sup> DM), in vitro neutral detergent fiber digestibility based on a 48-h incubation (NDFdom; g kg<sup>-1</sup> aNDF), in vitro true digestibility based on a 48-h incubation (IVTDOM; g kg<sup>-1</sup> DM), all corrected for the organic matter content of the residue, and relative feed value (RFV) (*n* = 315). Equation numbers refer to predictive equations presented in Table 1; Predictive equations of forage nutritive attributes from New York State (NYPEAQ) refers to equations developed by Parsons et al. (2013) and previously demonstrated to hold potential for use in Quebec (Wood et al., 2018).

Equation	Mean observ.	Mean prediction	R <sup>2</sup> †	RMSE	Slope coefficient (b)	SEb	P b = 1	Intercept coefficient (a)	SEa	P a = 0	d
Eq. [1]: aNDFom = 384 + (2.60 × AMAXHT) – (231 × AFRAC)	457	407	0.56	49.6	0.84	0.04	<0.001	116	17.2	<0.001	0.77
Eq. [2]: aNDFom = 356 + (1.79 × AMAXHT) + (0.93 × GMAXHT) – (203 × AFRAC)	457	418	0.65	44.4	0.91	0.04	0.019	76	16.0	<0.001	0.83
Eq. [3]: aNDFom = 352 + (6.88 × ASTAGE) + (1.34 × AMAXHT) + (1.05 × GMAXHT) – (205 × AFRAC)	457	417	0.67	42.9	0.92	0.04	0.023	74	15.3	<0.001	0.82
NYPEAQ: aNDFom = 125 + (224 × GFRAC) + (3.15 × AMAXHT)	457	414	0.55	50.1	0.76	0.04	<0.001	143	16.2	<0.001	0.79
Eq. [4]: ADFom = 159 + (1.29 × AMAXHT) + (0.83 × GMAXHT)	309	316	0.61	29.0	0.99	0.04	0.87	–4	14.2	0.76	0.86
Eq. [5]: ADFom = 155 + (5.42 × ASTAGE) + (0.93 × AMAXHT) + (0.93 × GMAXHT)	309	315	0.63	28.3	1.00	0.04	0.92	–4	13.6	0.75	0.87
Eq. [6]: ADFom = 144 + (0.90 × AMAXHT) + (0.77 × GMAXHT) + (22.6 × GGRP) + (0.10 × GDD5)	309	309	0.63	28.3	0.99	0.04	0.73	5	13.2	0.73	0.88
NYPEAQ: ADFom = 104 + (71.2 × GFRAC) + (2.54 × AMAXHT)	309	302	0.54	31.7	0.71	0.04	<0.001	96	11.3	<0.001	0.85
Eq. [7]: NDFdom = 1069 – (0.37 × GDD0) – (276 × AFRAC)	656	658	0.47	63.7	0.75	0.05	<0.001	160	30.1	<0.001	0.82
Eq. [8]: NDFdom = 829 – (1.64 × AMAXHT) – (0.22 × GDD0) + (250 × GFRAC)	656	665	0.57	57.2	0.84	0.04	<0.001	99	27.5	<0.001	0.86
Eq. [9]: NDFdom = 934 – (1.55 × AMAXHT) – (0.23 × GDD0) + (142 × GFRAC) – (112 × AFRAC)	656	658	0.57	57.6	0.83	0.04	<0.001	110	27.3	<0.001	0.86
Eq. [10]: IVTDOM = 1018 – (7.15 × ASTAGE) – (1.96 × AMAXHT)	805	857	0.56	35.5	0.96	0.05	<0.001	–13	40.9	0.75	0.69
Eq. [11]: IVTDOM = 1012 – (1.36 × AMAXHT) – (0.13 × GDD0) + (42.3 × GFRAC)	805	853	0.55	35.9	0.95	0.05	<0.001	–7	41.4	0.86	0.70
Eq. [12]: IVTDOM = 1016 – (0.90 × AMAXHT) – (0.73 × GMAXHT) + (40.5 × GGRP) – (0.16 × GDD5)	805	862	0.61	33.3	1.01	0.05	<0.001	–61	38.8	0.12	0.68
Eq. [16]: RFV = 165 – (1.17 × AMAXHT) + (89.9 × AFRAC)	131	145	0.56	19.3	0.77	0.04	<0.001	19	5.7	<0.001	0.82
Eq. [17]: RFV = 185 – (0.67 × GMAXHT) – (0.10 × GDD5) + (66.5 × AFRAC)	131	144	0.67	16.8	0.90	0.04	0.0039	2	5.2	0.70	0.85
Eq. [18]: RFV = 178 – (3.62 × ASTAGE) – (0.61 × AMAXHT) – (0.44 × GMAXHT) + (78.0 × AFRAC)	131	139	0.66	16.9	0.84	0.03	<0.001	13	4.9	0.007	0.87
NYPEAQ: RFV = 354 – (110 × GFRAC) – (0.13 × GDD0) – (1.09 × AMAXHT)	131	163	0.57	19.2	0.52	0.03	<0.001	47	4.3	<0.001	0.70

† R<sup>2</sup>, coefficient of determination; RMSE, root mean square error; AMAXHT, length in centimeters of the tallest alfalfa stem from the ground to the terminal bud once fully extended; GMAXHT, grass maximum height in cm length in centimeters of the tallest grass stem from the ground to the tip of the lastly emerged grass leaf; ASTAGE, stage of development of the most mature alfalfa stem alfalfa maximum stage based on Kalu and Fick (1981); GDD0, cumulated growing degree days base 0°C; GDD5, cumulated growing degree days base 5°C; AFRAC, alfalfa fraction of sample written as a decimal; GFRAC, grass fraction of sample written as a decimal; GGRP, grass fraction group, with group defined as the 0.2 interval closest to the GFRAC, written as a decimal (e.g., 0.6 or 0.8); d, index of agreement.

evaluated herein were developed with AFRAC and GFRAC being precisely determined; each botanical component being manually separated, dried, and weighed with exactitude using a scale. However, for the validation of the predictive equations with samples and data from commercial farms, AFRAC and GFRAC were estimated visually to simplify data collection by participants. They were the only variables that were collected or determined differently for the validation than for the development of the predictive equations. We thus hypothesized that the poor validation statistics were in part attributable to the large variation and errors in the visual estimations of AFRAC and GFRAC.

### Validation Using Visible Near-Infrared Reflectance Spectroscopy-Determined Alfalfa and Grass Proportions

To determine whether differences in the determination of AFRAC and GFRAC for the data used in the development and the validation of the predictive equations were the key reason for low R<sup>2</sup> values we developed and validated a VNIRS equation to estimate AFRAC values for samples collected on commercial farms. This VNIRS-based procedure was developed using the mixed alfalfa–grass samples from Part 1 of this study as well as samples from three other studies. Once the AFRAC values were

Table 3. Validation of equations developed in Quebec to predict nutritive attributes of mixed alfalfa–grass fields in the spring growth and the first summer regrowth and concurrent validation of comparable predictive equations developed for the spring growth in New York State. Values inputted into equations for grass fraction (GFRAC) and alfalfa fraction (AFRAC) were determined by visible near infrared reflectance spectroscopy (VNIRS). Data were collected from commercial farm fields located across 12 administrative regions of Quebec. These equations predict the following nutritive attributes: neutral detergent fiber (aNDFom; g kg<sup>-1</sup> DM), acid detergent fiber (ADFom; g kg<sup>-1</sup> DM), in vitro neutral detergent fiber digestibility based on a 48-h incubation (NDFdom; g kg<sup>-1</sup> aNDF), in vitro true digestibility based on a 48-h incubation (IVTDom; g kg<sup>-1</sup> DM), all corrected for the organic matter content of the residue, and relative feed value (RFV) (*n* = 315). Equations numbers refer to predictive equations presented in Table 1; Predictive equations of forage nutritive attributes from New York State (NYPEAQ) refers to equations developed by Parsons et al. (2013) and previously demonstrated to hold potential for use in Quebec (Wood et al., 2018).

Equation	Mean observ.	Mean prediction	R <sup>2</sup> †	RMSE	Slope coefficient (b)	SEb	P b = 1	Intercept coefficient (a)	SEa	P a = 0	d
Eq. [1]: aNDFom = 384 + (2.60 × AMAXHT) – (231 × AFRAC)	457	424	0.74	38.3	1.06	0.04	0.077	6	15.3	0.71	0.86
Eq. [2]: aNDFom = 356 + (1.79 × AMAXHT) + (0.93 × GMAXHT) – (203 × AFRAC)	457	434	0.79	34.3	1.08	0.03	0.015	–10	13.7	0.45	0.94
Eq. [3]: aNDFom = 352 + (6.88 × ASTAGE) + (1.34 × AMAXHT) + (1.05 × GMAXHT) – (205 × AFRAC)	457	433	0.80	33.1	1.07	0.03	0.026	–5	13.0	0.73	0.91
NYPEAQ: aNDFom = 125 + (224 × GFRAC) + (3.15 × AMAXHT)	457	431	0.69	42.0	0.91	0.01	0.035	65	15.2	<0.001	0.88
Eq. [6]: ADFom = 144 + (0.90 × AMAXHT) + (0.77 × GMAXHT) + (22.6 × GGRP) + (0.10 × GDD5)	309	309	0.64	27.9	0.99	0.04	0.80	4	13.0	0.78	0.88
NYPEAQ: ADFom = 104 + (71.2 × GFRAC) + (2.54 × AMAXHT)	309	307	0.58	30.4	0.74	0.04	<0.001	82	11.1	<0.001	0.87
Eq. [7]: NDFdom = 1069 – (0.37 × GDD0) – (276 × AFRAC)	656	680	0.58	56.6	0.99	0.05	0.76	–14	32.3	0.67	0.84
Eq. [8]: NDFdom = 829 – (1.64 × AMAXHT) – (0.22 × GDD0) + (250 × GFRAC)	656	684	0.67	50.1	1.03	0.04	0.45	–49	28.0	0.08	0.86
Eq. [9]: NDFdom = 934 – (1.55 × AMAXHT) – (0.23 × GDD0) + (142 × GFRAC) – (112 × AFRAC)	656	678	0.67	50.3	1.03	0.04	0.54	–39	27.8	0.16	0.87
Eq. [11]: IVTDom = 1012 – (1.36 × AMAXHT) – (0.13 × GDD0) + (42.3 × GFRAC)	805	857	0.55	35.9	0.97	0.05	0.54	–26	42.3	0.55	0.69
Eq. [12]: IVTDom = 1016 – (0.90 × AMAXHT) – (0.73 × GMAXHT) + (40.5 × GGRP) – (0.16 × GDD5)	805	861	0.61	33.5	1.02	0.05	0.67	–73	39.7	0.07	0.68
Eq. [16]: RFV = 165 – (1.17 × AMAXHT) + (89.9 × AFRAC)	131	138	0.69	16.1	0.93	0.04	0.06	2	4.9	0.69	0.89
Eq. [17]: RFV = 185 – (0.67 × GMAXHT) – (0.10 × GDD5) + (66.5 × AFRAC)	131	139	0.73	15.2	0.96	0.03	0.22	–2	4.7	0.65	0.92
Eq. [18]: RFV = 178 – (3.62 × ASTAGE) – (0.61 × AMAXHT) – (0.44 × GMAXHT) + (78.0 × AFRAC)	131	133	0.75	14.5	0.94	0.03	0.055	5	4.2	0.19	0.93
NYPEAQ: RFV = 354 – (110 × GFRAC) – (0.13 × GDD0) – (1.09 × AMAXHT)	131	154	0.63	17.6	0.57	0.02	<0.001	43	3.9	<0.001	0.77

† R<sup>2</sup>, coefficient of determination; RMSE, root mean square error; AMAXHT, length in centimeters of the tallest alfalfa stem from the ground to the terminal bud once fully extended; GMAXHT, grass maximum height in centimeters length in cm of the tallest grass stem from the ground to the tip of the lastly emerged grass leaf; ASTAGE, stage of development of the most mature alfalfa stem alfalfa maximum stage based on Kalu and Fick (1981); GDD0, cumulated growing degree days base 0°C; GDD5, cumulated growing degree days base 5°C; AFRAC, alfalfa fraction of sample written as a decimal; GFRAC, grass fraction of sample written as a decimal; GGRP, grass fraction group, with group defined as the 0.2 interval closest to the GFRAC, written as a decimal (e.g., 0.6 or 0.8); *d*, index of agreement.

determined using this approach, we calculated GFRAC by subtracting from 1 as participants collected samples mostly from field sections with minimal weed occurrence. This change also affected the GGRP variable and it was re-determined based on the new VNIRS-based GFRAC values, but not all values were affected because the GGRP variable only had four possible values (0.2, 0.4, 0.6, or 0.8). By inserting the VNIRS-determined AFRAC and GFRAC into the predictive equations to replace the values estimated visually by participants, while retaining all other field-collected data, the validation statistics were significantly improved for all predictive equations that used these variables (Table 3). The resulting R<sup>2</sup> values for linear regressions between observed and predicted aNDFom concentration and RFV met our minimum threshold of R<sup>2</sup> ≥ 0.75 for at least one of the three predictive equations.

For aNDFom, Eq. [2] and [3] met our minimum R<sup>2</sup> threshold with values of 0.79 and 0.80, and NRMSE values of 7.5 and 7.2%, respectively. Their slope remained significantly different from 1, while the intercept was not significantly different from 0 (Table 3). This condition, termed “rotation” by Gauch et al. (2003), indicated that predictions using these equations become less accurate as the forage matures and the aNDFom concentration increases (Fig. 1). The index of agreement (*d*), which indicate the goodness of fit of a model, was very good with values of 0.94 and 0.91 for Eq. [2] and [3], respectively. For RFV, Eq. [18] had an R<sup>2</sup> of 0.75 and a NRMSE of 11.1%, while the slope and intercept were not significantly different from the ideal, indicating the absence of bias (Fig. 2). The index of agreement (*d* = 0.93) was very good for this equation.

Our locally developed equations for all nutritive attributes generally had better validation statistics than the predictive equations

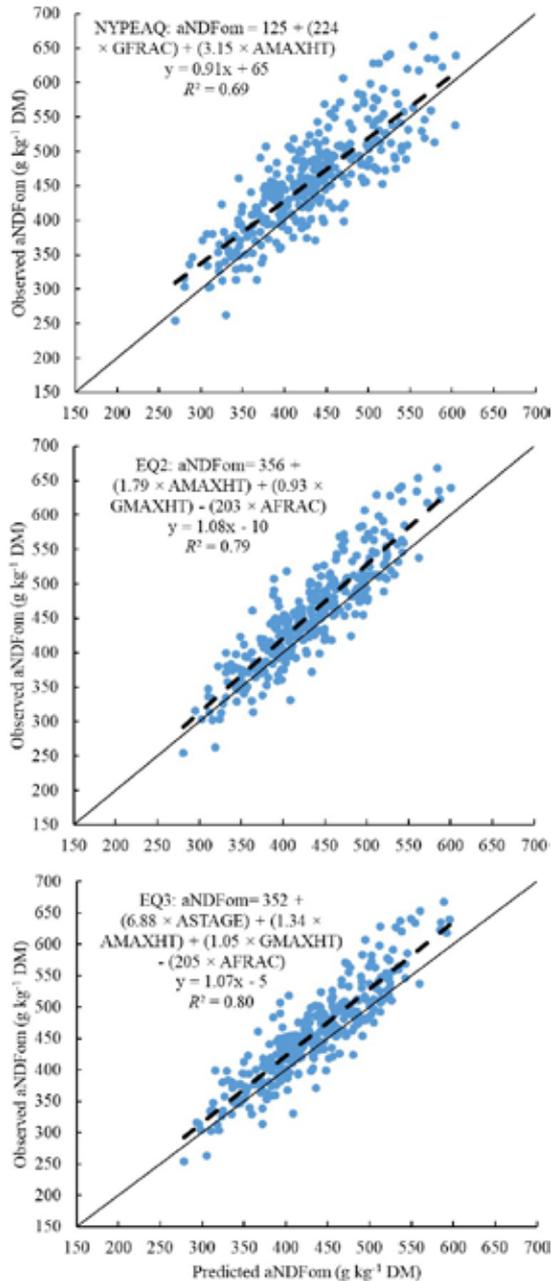


Fig. 1. Relationship between observed and predicted forage neutral detergent fiber concentrations of alfalfa–grass mixture samples collected on commercial farms in 12 regions of Quebec, Canada. Observed values were assayed with an  $\alpha$ -amylase and corrected for the ash content of the fiber residue (aNDFom), and predicted values were obtained by the predictive equations developed in Quebec (Eq. [2] and [3]) or by Parsons et al. (2013; NYPEAQ) in New York State. AFRAC, proportion of alfalfa within samples based on the dry matter (DM) weight written as a decimal (e.g., 0.1 or 0.6); AMAXHT, length in centimeters of the tallest alfalfa stem from the ground to the terminal bud once fully extended; ASTAGE, stage of development of the most mature alfalfa stem based on Kalu and Fick (1981); GFRAC, grass fraction within samples based on the dry matter weight written as a decimal (e.g., 0.1 or 0.6); GMAXHT, length in centimeters of the tallest grass stem from the ground to the tip of the lastly emerged grass leaf. The solid line indicates the ideal 1:1 relationship while the dotted line represents the regression line.

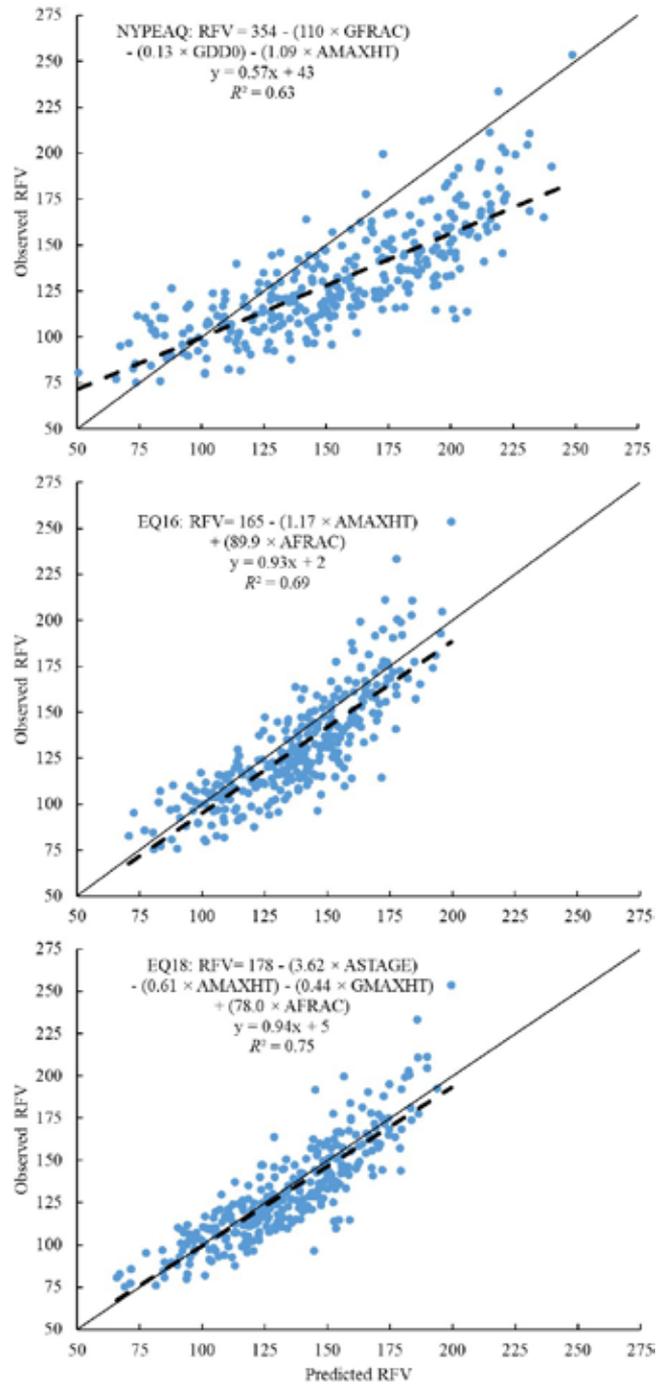


Fig. 2. Relationship between observed and predicted relative feed value (RFV) of alfalfa–grass mixture samples collected on commercial farms in 12 regions of Quebec, Canada. Predicted values were obtained by the predictive equations developed in Quebec (Eq. [16] and [18]) or by Parsons et al. (2013) (NYPEAQ) in New York State. AFRAC, alfalfa fraction within samples based on the dry matter (DM) weight written as a decimal (e.g., 0.1 or 0.6); AMAXHT, length in centimeters of the tallest alfalfa stem from the ground to the terminal bud once fully extended; ASTAGE, stage of development of the most mature alfalfa stem based on Kalu and Fick (1981); GDD0, cumulated growing degree days based 0°C; GFRAC, grass fraction within samples based on the dry matter weight written as a decimal (e.g., 0.1 or 0.6); GMAXHT, length in centimeters of the tallest grass stem from the ground to the tip of the lastly emerged grass leaf. The solid line indicates the ideal 1:1 relationship while the dotted line represents the regression line.

that Parsons et al. (2013) developed in New York State when using the data collected from the spring growth in commercial farm fields (Table 3, Fig. 1 and 2). Local predictive equations had greater  $R^2$  and  $d$  values, and smaller RMSE values. The use of VNIRS-determined AFRAC and GFRAC also improved the validation statistics of equations previously developed in New York State (Parsons et al., 2013), but their  $R^2$  values did not reach our minimum threshold of 0.75. Our results demonstrate that locally developed predictive equations based on samples collected from spring and first summer regrowth have better predictive capabilities for use on commercial farms across the province of Quebec than equations developed only for the spring growth in New York State. The poorer performance of equations previously developed in New York State to estimate NDF and ADF could be due to a range of factors including the fact that these equations were developed to estimate NDF and ADF values inclusive of residual ash, whereas the present study developed equations to estimate NDFom and ADFom. Other reasons include the fact that they were developed with a wider range of grass species in mixtures, and in a different geographical region.

The improvement in validation statistics resulting from the use of AFRAC and GFRAC determined by VNIRS rather than visually estimated was expected due to the importance of these two variables in predictive equations, as of all variables monitored they are the only estimated by users and hence most prone to error. Indeed, the magnitude of the coefficient value, which does not indicate importance but sensitivity in models, associated with either AFRAC or GFRAC was  $>200$  in the case of equations predicting aNDFom concentration and Eq. [7] and [8] predicting NDFdom (Table 3). With such coefficients, even a small change in the value of AFRAC or GFRAC would lead to significant changes in the predicted aNDFom or NDFdom values. For example, for Eq. [2] predicting aNDFom, the coefficient in front of the AFRAC variable is 203. In this case, a 0.05 change in the AFRAC value causes a  $10 \text{ g kg}^{-1}$  DM change in the estimated aNDFom concentration. Although this may seem relatively small, it would add to the error already inherently associated with the predictive equation.

A slight improvement in validation statistics was observed following the use of VNIRS-determined GGRP for Eq. [6] that predicts ADFom concentration (Table 3). This is not surprising as changes to GGRP values were minimal but also the value of the coefficient associated with this variable was small. For example, a change in GGRP value from 0.2 to 0.4 affected the predicted ADFom concentration by only  $4.5 \text{ g kg}^{-1}$  DM.

In the case of Eq. [11] predicting IVTDom, no improvement in the  $R^2$  value was observed following the use of VNIRS-determined GFRAC values (Table 3). This lack of improvement may be due to the fact that the variable was associated with a relatively low coefficient in the predictive equation (i.e., 42). The effect on Eq. [12], which included the GGRP variable, was also minimal, likely for the same reasons as mentioned earlier.

Although we observed improvement in the validation statistics for the majority of the predictive equations when using more precise VNIRS-determined GFRAC and AFRAC values, only three predictive equations met our a priori defined minimum threshold of  $R^2 \geq 0.75$  (Table 3). Some other factors must also be affecting our predictive equation results when used on commercial farms in a wide range of environments across the province

of Quebec. These factors may be associated with data collection, including differences in users' ability to measure certain variables (e.g., ASTAGE), errors in data recording, and inaccurate values of GDD0 and GDD5 due to the distance to the nearest meteorological station. Variations in field and soil management, grass species in the mixture along with errors in laboratory values due to the handling, storage, and shipping of samples could also have affected the results from the predictive equations.

## CONCLUSIONS

Equations for predicting aNDFom, ADFom, NDFdom, IVTDom, and RFV of the spring growth and the first summer regrowth of alfalfa–grass mixtures were successfully developed with  $R^2$  values  $>0.75$  and NRMSE  $<11.2\%$ . The validation of those predictive equations with field measurements during the spring growth from commercial farms was successful for aNDFom and RFV with  $R^2$  values  $>0.75$  and NRMSE  $<11.1\%$ . The validation for ADFom, NDFdom, and IVTDom was not as successful with  $R^2$  values between 0.61 and 0.67. The locally developed equations based on samples collected from spring through early summer growth of alfalfa–grass mixtures resulted in better validation statistics than similar equations developed to predict pre-harvest nutritive attributes of the spring growth of alfalfa–grass mixtures in New York State. The success of these predictive equations improved with a more precise determination of the contribution of alfalfa or grass to forage DM yield. The predictive equations are based on the field measurements of relatively simple variables and will help forage producers in determining optimal harvest time for alfalfa–grass mixtures. These equations although depend on the determination of precise contribution of alfalfa or grass to forage DM yield, field variables that are difficult to estimate visually for untrained users. It might thus be preferable to do a hand separation of samples to determine with more precision this important variable.

## SUPPLEMENTAL MATERIAL

Two tables are provided as supplemental material. The first table provides descriptive statistics for alfalfa–grass mixture samples collected at three contrasted sites (Sainte-Anne-de-Bellevue, Levis, and Normandin) in Quebec, Canada, and used to develop predictive equations for forage nutritive attributes (Part 1). The second table provides descriptive statistics for alfalfa–grass mixture samples collected in commercial fields in 12 administrative regions of Quebec, Canada, and used to validate predictive equations for forage nutritive attributes (Part 2).

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