Predictive equations of forage nutritive value for use under Québec's environmental conditions

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Abstract

The primary objective of this project was to determine if predictive equations developed in the United States can be used in Québec to predict the pre-harvest forage nutritive value of alfalfa-grass mixtures in the field. These equations use relatively simple measurements that can quickly be determined in the field by an agricultural producer or an agronomist to predict important variables including the neutral detergent fibre (NDF) concentration and thus allow for the harvest of forages at an optimal stage. The aim was to determine if equations developed in New York State for the first spring growth can be used in Québec and also to develop equations specific for Québec that could be used to determine a larger number of attributes describing forage nutritive value for both the spring growth and the first summer regrowth. Results demonstrate that equations developed in New York State for the first spring growth have the potential to be used in Québec to predict the NDF assayed with α–amylase (aNDF) and acid detergent fibre (ADF) concentrations of alfalfa-grass mixtures. New equations developed in Québec can be used to predict forage nutritive value of both the spring growth and the first summer regrowth. If results obtained were excellent for some nutritive attributes including aNDF and ADF concentrations, it was not the case for others including crude protein concentration. Newly developed equations were finally validated using samples collected on commercial farms from 12 regions of the Province of Québec. This validation demonstrated that some equations can be used across the Province to predict the aNDF concentration and the Relative Feed Value (RFV) if the alfalfa:grass proportions can be precisely determined. This project provides Québec agricultural producers with a simple tool to determine when to harvest their forages.

Résumé

L'objectif principal de ce projet était de déterminer si les équations de prédiction développées aux États-Unis peuvent être utilisées au Québec pour prédire au champ la valeur nutritive de mélanges luzerne-graminées. Ces équations utilisent des mesures simples qui peuvent être rapidement déterminées en champ par un producteur agricole ou un conseiller/agronome pour prédire des variables importantes telle la concentration en fibres insolubles au détergent neutre (NDF) et ainsi permettre la récolte des fourrages à un stade optimal. Dans un premier temps, le but était de déterminer si des équations développées dans l'état de New York pour la croissance printanière pouvaient être utilisées au Québec, et dans un deuxième temps, de développer des équations spécifiques pour le Québec afin de déterminer la valeur nutritive des fourrages pour les deux premiers cycles de croissance et pour un nombre plus élevé de variables de valeur nutritive. Les résultats démontrent que les équations développées pour la croissance printanière dans l'état de New York ont le potentiel d'être utilisées au Québec pour prédire certaines variables incluant les concentrations en NDF et en fibres insolubles au détergent acide (ADF). Cependant, les équations développées au Québec permettent non seulement de prédire la valeur nutritive lors du premier cycle de croissance mais aussi lors du deuxième. Si les résultats sont excellents pour certaines variables comme les concentrations en NDF et ADF, ce n'est pas le cas pour d'autres incluant la concentration en protéines brutes. Les équations développées au Québec ont finalement été validées en utilisant des échantillons prélevés sur des fermes commerciales dans 12 régions de la Province. Cette validation a démontré que certaines équations peuvent être utilisées à travers la Province pour prédire la concentration en NDF et la « Relative Feed Value » (RFV) si les proportions luzerne:graminées sont déterminées avec précision. Ce projet fournit un outil simple permettant aux producteurs agricoles québécois de déterminer le moment opportun pour récolter leur fourrage.

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Contribution of Authors

This thesis has been written in standard format. This research was designed by Philippe Seguin, Gaëtan Tremblay, Gilles Bélanger, Julie Lajeunesse, Annie Claessens, Robert Berthiaume, and Huguette Martel. The protocol of the research project was created with contributions from the aforementioned individuals as well as the candidate. The candidate conducted all field data and sample collection for the Sainte-Anne-de-Bellevue research site, performed some chemical analysis for nutritive attributes of interest, compiled data and performed statistical analysis as well as wrote the thesis while under the direct supervision of Philippe Seguin.

Gaëtan Tremblay, co-supervisor to the candidate, and Gilles Bélanger of Agriculture and Agri-Food Canada (AAFC) in Québec City provided advice throughout the project. They provided oversight to the research fields located in Québec city as well as supervision of the candidate and AAFC staff during the chemical analysis performed in the laboratory of the AAFC research and development centre in Québec city. Julie Lajeunesse managed and supervised the research fields at the AAFC research farm in Normandin. Annie Claessens conducted proof of concept research before the project was undertaken in full by the candidate, although no data or analysis from the proof of concept was used in the final results of this project. Huguette Martel of MAPAQ, Direction régionale de l'Estrie, and Robert Berthiaume of Valacta, coordinated and supervised the work performed by the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) and Valacta advisors.

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1. General Introduction

1.1. Rational for the project

Québec has a large number of animal producers who rely on forage crop production. To increase profitability, producers must either increase end product price or reduce costs. With the dairy industry, the system is heavily regulated with respect to output product pricing, meaning that the individual producer has little say over the price received for milk components. Beef producers sell into a free market but due to its nature, individual producers find themselves in similar positions as dairy producers, unable to increase end product pricing based on what the local markets have set. This leaves cutting costs as the main option of increasing profitability. For both production systems, feed represents both one of the largest costs incurred and a major factor in the amount and quality of output that the animals produce. Ruminant dairy and meat production rely on a combination of forages, grains and other inputs to meet the nutritional demands needed to produce milk, muscle, and fat. The price of grains in recent years has been quite significant, encouraging a shift in ruminant diets towards more forages, however, the variation in forage nutritive value can lead to an uncertain impact for producers requiring highly nutritious, consistent feed inputs.

Forage nutritive value is affected by numerous factors like species, environmental effects, harvest date, and storage process (Van Soest, 1985). The timing of the harvest date is particularly important as producers must compromise between the feed nutritive value and the biomass produced. The optimal concentration of neutral detergent fibres (NDF) is 45% DM for alfalfa silage and 50% DM for grass silage fed to highly productive dairy cows (Cherney et al., 1994).

Methods for predicting the optimal time of harvest can be done through laboratory analyses but the process can be lengthy, leading to a missed window for harvesting at the optimal time. An in-field method of forage nutritive value prediction, known as "Predictive Equations of Alfalfa Quality, PEAQ" and created by Hintz and Albrecht (1991), can be rapid, relatively accurate, economical, and easy to use by producers. These predictive equations can provide the necessary information when deciding a harvest date to ensure that forage falls into the narrow range of optimal forage nutritive value. Such equations use simple measurements that can be collected directly in the field, including for example maximum plant height and stage of development of plants, as well as meteorological data in some cases (e.g., Hintz and Albrecht, 1991; Parsons et al., 2006a, 2006b). The PEAQ equations have since been modified by Parsons et al. (2006b, 2013) for New York State to predict forage neutral detergent fibre assayed with a heat stable α–amylase (aNDF) and acid detergent fibre (ADF) concentrations, relative feed value (RFV), and less accurately, *in vitro* NDF digestibility (NDFd), and relative feed quality (RFQ) in alfalfa-grass mixtures before their harvests. These mixed forage predictive equations could be very useful for Québec producers; however, they must be validated locally before their use can be recommended.

By adapting the equations developed by Parsons et al. (2006b, 2013) to Québec's environment, producers would have a tool that fits the need for a fast, relatively accurate and economical predictor of their forage nutritive value in field allowing them to harvest at the optimal time, and therefore reducing the uncertainty in these values. This can provide greater certainty in diet formulation, allowing decreased reliance on grains while still producing to similar or greater levels of output. Overall, this can lead to a decrease in costs and, potentially, an increase in profitability for animal producers.

1.2. Objectives and hypotheses

Objective 1: To evaluate whether predictive equations of forage nutritive attributes for mixed grass-alfalfa fields developed in New York State (NYPEAQ) by Parsons et al. (2006b, 2013, 2014) for the spring growth are valid under Québec's environmental conditions.

Hypothesis: We defined *a priori* that the relation between nutritive attributes estimated using predictive equations developed in New York State and those determined from field samples analyzed in the laboratory must meet a minimum threshold of an $r^2 \geq 0.75$ to be considered valid for the Province of Québec. We hypothesize that some of the equations developed in New York State will be valid for use locally.

Objective 2: To determine whether it is possible to create equations specifically for Québec that could be used to predict the nutritive value of alfalfa-grass mixtures at the spring growth and the first summer regrowth. The goal is to develop equations for forage yield and for as many nutritive attributes as possible including neutral detergent fibre assayed with a heat stable α–amylase corrected for the ash content of the fibre residue (aNDFom), acid detergent fibre corrected for the ash content of the fibre residue (ADFom), *in vitro* NDF digestibility corrected for the ash content of the fibre residue (NDFdom), relative feed value (RFV), relative feed quality (RFQ), crude protein (CP), and *in vitro* true digestibility of DM corrected for the ash content of the fibre residue (IVTDom).

Hypothesis: Again, we defined *a priori* that the relation between nutritive attributes estimated using newly developed predictive equations and those from field samples analyzed in the laboratory must meet a minimum threshold of a $R^2 \geq 0.75$ to be considered valid for the Province of Québec. We hypothesize that we will be able to successfully develop equations to predict several attributes of importance, although the predictions will be less accurate than previous developed equations (e.g., PEAQ) given our equations will be developed for both the spring growth and the first summer regrowth.

Objective 3: To determine if the newly developed equations in objective 2 can be used to predict nutritive attributes of mixed alfalfa-grass field on commercial farms across the Province of Québec with data being collected by a range of individuals (i.e., agronomists). This validation step will provide insight into how the equations perform under increased variability of environments and expertise of those sampling. This will help during the knowledge transfer step for commercial application.

Hypothesis: We hypothesize that few of the newly developed equations in objective 2 will show a relation between nutritive attributes estimated using predictive equations and those from field samples analyzed in the laboratory that meet our minimum threshold of $r^2 \geq 0.75$. The reason is that as the number of individuals collecting data and the environments in which they are collected increase, the quality of the data collected decreases, and the variability increases.

2. Review of Literature

2.1. Forage crops

Forage crops are an important part of Canadian agriculture. According to the FAO, forages are seeded on over 36 million hectares of land and are used in animal production industries such as dairy, beef, sheep, and goat productions (FAO, 2010). Forages are important in ruminant diets to ensure good health, increased production and longevity, all of which lead to greater economic benefits for producers. The predominant forages species used in Québec are alfalfa and timothy, for hay and silage production for all ruminant production systems including dairy, beef, sheep, and goat producers. Tall fescue, while not as popular in Québec as timothy or alfalfa, is considered to be an alternative grass to timothy under the condition of climate change. As new cultivars with increased winter hardiness become available, there may be an increase in interest for this species.

2.1.1. Alfalfa

Alfalfa is a perennial forage legume that is one of the oldest cultivated crops as well as one of the most widely grown crops throughout the world (Michaud et al., 1988). There are three main species in usage; *Medicago sativa* L., *Medicago falcata* L., and *Medicago media* Pers. (Michaud et al., 1988). It is well known for its symbiotic relationship with the nitrogen fixing bacteria *Sinorhizobium melitoli*, which leads to greater forage protein concentration and yields. Because of its high nutritive value, palatability for ruminants, and its ability to recover from multiple cut systems, alfalfa is useful in silage and hay making, but also in pasture when grown in mixtures with grasses.

Alfalfa stem height varies according to cultivars and environmental conditions it is sowed in. It is a bunch type forage with stems emerging from the crown of the plant, which are initially above ground but eventually pulled underground by contracting cells (Teuber and Brick, 1988). Initial leaf is unifoliate with remaining leaves being trifoliate or multi-foliate depending on the cultivar. Leaves grow pinnately whether trifoliate or multi-foliate. The leaves are long, narrow, oblong shaped and larger in the center than the ends along with serrations appearing half way down the leaf. The inflorescence is a raceme type with generally purple flowers. The root system is a large tap root that can reach depths of 2-4 m on average. These deep roots are a way to combat drought conditions.

Alfalfa germination can occur over a wide range of temperatures (5 to 30°C) but is optimum between 19 and 25°C (Fick et al., 1988). Optimum temperature for plant growth ranges from 15 to 27°C depending on the season period and the cultivar, younger stages requiring higher temperatures and more mature stages requiring lower temperatures. Alfalfa requires well drained soils and a $pH > 6.5$ to ensure an optimal environment for nitrogen fixing bacteria for nodules formation. Poorly drained soils can lead to anaerobic environment, which will inhibit root growth and beneficial microbial populations. Soils with a $pH < 6.5$ can inhibit beneficial microbial populations, affecting nodulation formation and therefore nitrogen fixing capabilities of the plant.

2.1.2. Timothy

Timothy (*Phleum pratense* L.) is a cool season perennial bunch type grass that is very popular in Québec due to its winter hardiness. It is widely used for silage and hay production (Berg et al., 1996), specifically in a two-cut system due to its slow regrowth. This allows producers to harvest in the spring and summer to store for winter feeding. Timothy can reach heights of up to 100 cm. It has a membranous ligule, no auricles and a split sheath (Berg et al., 1996). New tillers emerge from buds found at the base of the corm, a lower node that has enlarged into a bulb like storage organ. Tillers grow intravaginally, this leads to the vertical growth commonly seen with bunch type grasses. Once these tillers reach a certain maturity, their own corms will be formed, replacing older corms and the cycle will continue. Leaves can reach up to 50 cm in length, up to 1 cm wide and are pointed at the end; the inflorescence is panicle and cylindrical with lengths between 5-10 cm. The root system is fibrous and shallow and non-sod forming (Berg et al., 1996), meaning that droughts will have a large impact on timothy. Seed germination occurs between 5 and 25°C, with higher temperatures resulting in faster germination rates (Charlton et al., 1986). Growth is optimal when temperatures are 21°C during the day and 15°C during the night (Smith, 1972).

2.1.3. Tall fescue

Tall fescue (*Festuca arundinacea Schreb.*) is a cool season perennial bunch type grass that is very popular in the United States. It is predominately used for hay and silage and is best known for its wide adaptability to many environmental and soil conditions and its ability to hold nutritional value, allowing stockpiling to occur for winter feeding. It is also known for the fact that some cultivars have a symbiotic relationship with an endophyte that enhances tall fescue's ability to tolerate environmental stresses but also can lead to animal toxicity when ingested (Hoveland, 2009), however, this can be prevented by using non infected seeds. Tall fescue stems are erect and tall. They have a membranous ligule, a smooth leaf sheath, auricles can either by short and hairy or not present and the collar is hairy. Leaves can reach up to 60 cm in length and can be up to 12 mm wide. The panicles range from 10 to 50 cm in length and can be either tightly or loosely held together (Terrell, 1979). Tillering in tall fescue occurs intravaginally, as with other bunch type grasses (Sleper and West, 1996). Tall fescue continuously produces new tillers to replace old tillers and increase competitiveness (Nelson, 1996). Rhizomes are generally short or not present, however, due to the wide genetic diversity of tall fescue and endophyte interaction, this may not always be the case (Sleper and West, 1996). Seed germination occurs over a wide range of temperature: 8 to 33°C (Wolf et al., 1979). Temperatures for plant growth vary depending on the stage of development and whether it is day, which requires warmer temperatures, or night, which requires cooler temperatures. In the case of seedling growth for better forage nutritional quality and tillering, the optimal temperature is from 10 to 20°C, while in the case of relative plant growth the optimal temperature is 22 to 27°C (Wolf et al., 1979).

2.2. Nutritive attributes in forages

In the case of ruminant production, whether for meat, milk or wool, forages or roughage represent an important feed to ensure a healthy and economical diet. The use of forages takes advantage of the evolutionary development of the ruminant's ability to break down components unavailable to most monogastrics (Pond, 2005). This breakdown occurs due to the fermentation process by the vast microbial populations found in rumens, as well as the mechanical movements of the rumen itself. Producers must be careful as not all forages are of the same nutritional quality and therefore will not provide the same level of nutrition (Collins and Fritz, 2003). This can lead to juggling of ingredients in ruminant diets to ensure the proper blend between forages, grains and other inputs for optimal production. As forages generally cost less than other inputs, it is important to get the highest nutritive value possible whether the producer is purchasing from elsewhere or growing their own.

Traditionally, when discussing forage nutritive value for ruminants, the main attributes taken into account most often include crude protein (CP), neutral detergent fibre assayed with a heat stable α–amylase (aNDF), and acid detergent fibre (ADF) (Collins and Fritz, 2003). However, more recently it has been found that a better analysis of forage nutritive value should include *in vitro* true digestibility of dry matter (IVTD) and *in vitro* NDF digestibility (NDFd). Relative feed value (RFV) and relative feed quality (RFQ) are also used as indices to compare forage quality in various regions of North America.

2.2.1. Neutral detergent fibre assayed with a heat stable α–amylase (aNDF)

The aNDF predominately encompasses some of the structural carbohydrates most commonly found in forages, cellulose and hemicellulose as well as lignin and other minor components (Pond et al., 2005). The percentage of plant matter that is aNDF can be identified by using Van Soest's neutral detergent extraction. The aNDF concentration in forages is dependent on many variables including the species, cultivars, growth rate, stage of development, leaf/stem ratio, meteorological conditions, soil conditions, and available nutrients (Marten et al., 1988). With respect to ruminant diets, aNDF is used as an indicator of feed intake.

2.2.2. Acid detergent fibre (ADF)

The ADF is primarily made up of lignin and cellulose, along with other minor structural components (Pond et al., 2005). It can be analysed by the Van Soest acid detergent extraction process. As with aNDF, forage ADF concentration varies depending on numerous variables. With respect to ruminant diets, ADF is used as an indicator of feed digestibility.

2.2.3. Crude protein (CP)

The CP can be estimated using the Kjeldahl analysis method, which determines the total nitrogen concentration. This value is then multiplied by 6.25 to get the CP value (CP, % DM = N, % DM × 6.25) (Pond et al., 2005). The reason for the use of the 6.25 conversion factor is that protein is, on average, made up of 16% nitrogen (100/16 = 6.25). This method takes into account all nitrogen within the sample regardless of its availability.

2.2.4. Relative feed value (RFV)

The RFV is an index for alfalfa based hays and silages and is made up of two components: the digestible dry matter (DDM) and the dry matter intake (DMI). These two components are multiplied together and then divided by 1.29 (Jeranyama and Garcia, 2004). The index was created such that full bloom alfalfa containing an aNDF concentration of 53 % DM and an ADF concentration of 41 % DM was the base of the index at RFV=100. When alfalfa based feeds are graded on this system and have a score higher than 100, it indicates that the feed has greater digestibility and intake potential than the base feed used to make the index. While the simplicity of use and understanding of this index is welcome, it also creates limitations such as: it is only based on ADF and aNDF and therefore does not take into other important nutritive attributes like CP; it also lacks the complexity to be used for other forages such as predominately grass based feeds; and it should not be used when creating rations as it does not take into account full nutritional information of the feed.

$$\mathrm{DDM} = 8.89 - (0.779 \times \%\ \mathrm{ADF})$$

$$\mathrm{DMI} = \left(\frac{120}{\%\ \mathrm{aNDF}}\right)$$

$$\mathrm{RFV} = \frac{(\mathrm{DDM} \times \mathrm{DMI}))}{1.29}$$

<u>2.2.5. Relative feed quality (RFQ)</u>

The RFQ is a more nuanced approach than RFV when estimating nutritional quality of forages as the formula for calculating it changes depending on whether the feed is predominately alfalfa or grass and accounts for the differences in digestibility of fibres caused by changes to the numerous variables (e.g. weather, species, and cutting) that occur during growth. We can estimate the RFQ by using DMI and total digestible nutrients (TDN) (Jeranyama and Garcia, 2004). The method for identifying TDN and DMI and then RFQ is as followings (Undersander et al., 2013):

$$\mathrm{RFQ} = \frac{\big((\mathrm{DMI}, \%\ \mathrm{of\ BW}) \times (\mathrm{TDN}, \%\ \mathrm{of\ DM})\big)}{1.23}$$

<u>For TDN</u>:

$$TDN = [(0.93 \times CP) + (0.97 \times (EE - 1) \times 2.25) + (NFC \times 0.98) + \left((NDF - NDFCP) \times \left(\frac{NDFd_{adjusted}}{100}\right)\right)] - 7$$

Where:

CP = crude protein (% of DM)

EE = ether extract (% of DM)

NFC = non fibrous carbohydrates (% of DM) $= 100 - (CP + NDF_{adjusted} + ash + EE)$

NDF = neutral detergent fibre (% of DM)

NDFCP = neutral detergent fibre insoluble crude protein (% of DM)

$NDF_{adjusted}$ = nitrogen free NDF = NDF – NDFCP, also estimated as NDFn = NDF × 0.93

<u>For DMI</u>:

$$DMI = [120/(NDF + (NDFd - 45) \times \frac{0.374}{1350} \times 100)]$$

Where:

NDF = neutral detergent fibre (% of DM)

NDFd = neutral detergent fibre digestibility after a 48h *in vitro* incubation with rumen fluid (% of NDF)

45 = average value for fibre digestibility of alfalfa and alfalfa/grass mixtures.

2.2.6. *In vitro* true digestibility of dry matter (IVTD)

The IVTD is an analytical method used to simulate the digestive system of the animal in order to identify the true digestibility of a feed or forage under such a system (Boisen, 2000). The goal is to give a repeatable and better estimate of the digestibility of various feedstuffs a ruminant may be given. The method for IVTD is a modified Tilley and Terry method (Tilley and Terry, 1963). Initial incubation in rumen fluid occurs over a 48-hour period but in the following step the residues are run through a neutral detergent extraction process instead of digesting further in pepsin and a weak acid (Van Soest, 1994). By putting the incubation residues through the neutral detergent extraction, the final residue will consist only of undigested plant cell wall material, which provides a better measurement of the digestibility of the feed (Van Soest, 1994).

2.2.7. Neutral detergent fibre digestibility (NDFd)

The NDFd is a measure of how digestible the NDF is in the rumen, this varies depending on forage, stage of development, and growth conditions for example. The NDFd has also been linked to animal performance, with greater NDFd resulting in greater performance. It is measured as a percentage of NDF or in terms of g kg^{-1} of NDF (Oba and Allen, 1999). It can be measured using the residual material leftover from the IVTD method. The residual material in the IVTD method is undigested NDF components. By subtracting undigested NDF weight from the initial NDF weight result, provides the weight of the NDF that had been digested. By further dividing this number by the initial NDF weight and multiplying by 100, we would get the % of NDF that is digestible (Goeser and Combs, 2009).

2.3. Predicting forage nutritive attributes

The ability to provide quick, accurate, easily executed, and repeatable estimations is desired by research and commercial interests. This interest as well as the growing knowledge of forages, their growth patterns, and variables that affect their component composition has led to new methods of predicting forage nutritive value. Traditionally, methods used to determine forage nutritive value were based on laboratory analysis, meteorological data, or morphological information gathered by labour intensive means. However, this changed in 1991 with the development of Predictive Equations of Alfalfa quality (PEAQ), which uses simple morphological measures to estimate nutritive value (Albrecht and Hintz, 1991).

2.3.1. PEAQ in Wisconsin

Predictive equations of alfalfa quality were originally created in Wisconsin in 1991 by Albrecht and Hintz. Their objective was to identify a method to predict in-field alfalfa aNDF concentration in pure alfalfa fields that was fast and as accurate as possible, in order to harvest alfalfa at the optimal time (Albrecht and Hintz, 1991). This was in comparison to methods in use at the time such as laboratory analysis using Visible Near Infrared Reflectance Spectroscopy (VNIRS), predictions based on meteorological data (which may not be easily available), or by time consuming manual labour methods [mean stage by count (MSC) or by weight (MSW), Kalu and Fick, 1981]. Hintz and Albrecht (1991) gathered data from various morphological variables and a full list can be found in Table 2.1. They then compared this data to their analysis of the samples for neutral detergent fibre assayed with a heat stable α–amylase (aNDF), acid detergent fibre (ADF), acid detergent lignin (ADL), and crude protein (CP). They performed linear and multiple regression analyses to test and create single, double and tertiary variable equations and identified those with the highest R^2 and lowest root mean square error (RMSE). Equations were narrowed based on confidence in prediction and ease of use for producers, consultants and researchers. The equations that they generated can be found in Table 2.2. The two variables of importance are the height of the tallest alfalfa stem (AMAXHT) and the morphological stage of development of the most mature alfalfa stem (AMAXSTAGE) present in each sample. This data is easy to gather and values can thus be quickly determined.

2.3.2. PEAQ in the rest of the United States

The potential economic benefits garnered from and the ease of use and relatively accurate estimates of PEAQ in Wisconsin lead to interest from researchers in other states, notably Ohio, California, Pennsylvania, and New York, who tested whether these equations were as accurate in their prediction when working under different environmental conditions. It was found that these equations could be used in other states, despite their differing environmental conditions; however, increased bias was observed when the equations were used outside of Wisconsin (Sulc et al., 1997). In the case of PEAQ in New York, Parsons et al. (2006a) confirmed that the height of the tallest alfalfa stem in the sample area (AMAXHT) was of more importance than the morphological stage of the most mature stem in the sample area (AMAXSTAGE). The resulting predictive equations using only AMAXHT as a variable were only slightly less accurate than those that used both AMAXHT and AMAXSTAGE.

2.3.3. Predicting aNDF concentration of alfalfa-grass mixtures in New York

Predictive equations were considered to be a step forward on the path to produce a more consistent quality animal feed. However, their use was limited to fields sown with pure alfalfa, a practice uncommon in the northeastern U.S. (Parsons et al., 2006b). The creation of equations to predict forage aNDF concentration for fields sown with grass-alfalfa mixtures, the common practice in the area, was done by Parsons et al. (2006b). This was more difficult than for the original PEAQ equations as the new equations needed to accurately predict two forages with different developmental patterns and morphological characteristics as well as little knowledge on the grass-alfalfa interaction when grown together. The variables of importance for this study can be found in Table 2.3. Parsons et al. (2006b) found that they were able to predict forage aNDF concentration with varying degrees of confidence, depending on the variables used. The key variables for the equations with the greatest confidence were the accurate estimation of grass fraction within the sample, the height of the tallest alfalfa stem in the sample and either the accumulated growing degree days based on 5°C or the morphological stage of the most mature grass tiller using the simplified morphological system of Moore and Moser (1995). It was also found in this research that data from one year should not be used in a predictive equation using meteorological data from a previous year as this will increase inaccuracies. The equations of interest (NYPEAQ) developed by Parsons et al. (2006b) can be found in Table 2.4.

2.3.4. Expanding predictive equations (NYPEAQ) to RFV and RFQ of alfalfa-grass mixtures in New York

The use of aNDF concentration of forages is a highly used measurement by producers and nutritionists alike when evaluating forages. However, depending on the region, other variables can also be used to assess forage nutritive value. As previously mentioned, RFV and RFQ are increasingly used indexes of forage nutritive value that encompass some or many nutritive attributes of importance when discussing forage nutritive value. Being able to predict these indexes would provide ease of comparison for producers in areas that rely on these measurements. Due to the nature of NDFd and its increasing use when creating feed rations, the ability to predict this variable would also be important to producers. With this in mind, Parsons et al.(2013) selected samples from the 2006b study done by Parsons et al. (2006b) on creating a predictive equation of aNDF concentration in mixed alfalfa-grass fields to be used in a new study to see if it was possible to predict RFV and RFQ of mixed alfalfa-grass fields. The criteria for the samples to be used in this analysis was that they were representative of both years of the previous study, the three grass species used and that the alfalfa portion of the sample contained 30-50% aNDF while the grass portion contained 40-60% aNDF. The variables used were from the Parsons et al. (2006b) study and can be found in Table 2.3. The chemical analyses performed were the same as those in 2006b as well. The resulting predictive equations for aNDF, ADF, RFV, NDFd, and RFQ were initially created using only plant morphological variables and had mixed predictive capabilities. While forage aNDF concentration was predicted quite accurately using only morphological characteristics, the other nutritive attributes were predicted with less accuracy.

Table 2.1. – Description of alfalfa morphological traits that were measured and utilized in linear and multiple regression analyses for Predictive Equations of Alfalfa Quality (PEAQ) in Wisconsin.

Character	Description
LEAF	Percent of total plant mass present as leaf and petiole.
STEM	Percent of total plant mass present as stem and stipule
REPRO	Percent of total plant mass present as reproductive structures.
MSW	Mean stage of morphological development based on plant mass present in each stage category.
MSC	Mean stage of morphological development based on number of stems in each category.
C50	Maximum morphological stage of development reached or exceeded by 50% of stems.
W50	Maximum morphological stage of development reached or exceeded by stems constituting 50% of the sample mass.
C25	Maximum morphological stage of development reached or exceeded by 25% of stems.
W25	Maximum morphological stage of development reached or exceeded by stems constituting 25% of the sample mass.
AMAXSTAGE	Morphological stage of development of the most mature stem present in each sample.
MHW	Mean steam height (cm), weighted for plant mass.
MHC	Mean stem height (cm).
AMAXHT	Height (cm) of the tallest stem present in each sample.
MNW	Mean node number, weighted for plant mass.
MNC	Mean node number.

Table 2.2. – Final PEAQ regression equations to predict forage crude protein (CP) and fibre concentrations (aNDF, ADF, and ADL) in % of DM based on the height of the tallest alfalfa stem (AMAXHT, cm) and the morphological stage of development of the most mature alfalfa stem present in the sample (AMAXSTAGE) (Hintz and Albrecht, 1991).

Regression Equations	R^2	RMSE
CP = 307.1 – (0.9 × AMAXHT) – (8.9 × AMAXSTAGE)	0.74	21.7
aNDF = 168.9 + (2.7 × AMAXHT) + (8.1 × AMAXSTAGE)	0.89	26.2
ADF = 115.7 + (2.1 × AMAXHT) + (7.9 × AMAXSTAGE)	0.88	22.0
ADL = 15.8 + (0.5 × AMAXHT) + (2.5 × AMAXSTAGE)	0.84	6.5

Table 2.3. – Descriptions of the variables of importance for Parsons et al. (2006b) equations of prediction (NYPEAQ) for forage nutritive value in alfalfa-grass mixtures.

Variable	Description
ALTD	Difference between altitudes of weather station and field (m)
ALTF	Altitude of sample field (m)
ALTWS	Altitude of sample station (m)
GCANOPY	Height of the grass canopy in the sample area (cm)
GDD0	Accumulated growing degree days, base 0°C
GDD5	Accumulated growing degree days, base 5°C
GFRAC	Actual fraction of grass in the sample (kg kg^{-1} DM)
GGRP	Grouped fraction of grass in the sample (kg kg^{-1} DM)
GMAXHT	Height of the tallest grass plant in the sample area (cm)
GMAXNDX	Developmental stage of most mature grass tiller in the sample area
GMAXSTG	Developmental stage of most mature grass tiller in the sample area using a simplified system
GSPECIES	Major grass species in each sample area
DOY	Day of the year
AMAXHT	height of the tallest alfalfa stem in the sample area (cm)
AMAXSTAGE	Morphological stage of development of the most mature alfalfa stem in the sample area
TIME	Time of sample (decimal hours)

Table 2.4. – Final equations of interest from (NYPEAQ) Parsons et al. (2006b) for predicting aNDF concentration (% of DM) in mixtures of alfalfa-grass based on the height of the tallest alfalfa stem (AMAXHT, cm), the actual grass proportion (GFRAC, g kg^{-1} DM)), the growing degree-days (5°C basis, GDD5), the grouped fraction of grass in the sample based on 20% intervals (GGRP), and the morphological stage of development of the most mature grass stem (GMAXSTG) present in the sample.

Equations of interest	R^2	RMSE
aNDF=87.1 + (3.2 × AMAXHT) + (313 × GFRAC)	0.89	30.1
aNDF=91.2 + (2.1 × AMAXHT) + (290 × GFRAC) + (0.28 × GDD5)	0.94	22.4
aNDF=97.9 + (2.1 × AMAXHT) + (269 × GGRP) + (0.29 × GDD5)	0.91	26.7
aNDF=106 + (2.6 × AMAXHT) + (264 × GGRP) + (4.3 × GMAXSTG)	0.88	31.1

3. Materials and Methods

3.1. Objective 1. Evaluating the potential of predictive equations of forage nutritive attributes developed in New York State for use in Québec.

3.1.1. Field description

Plots of alfalfa and grass mixtures were seeded in May 2014 at the following locations: Sainte-Anne-de-Bellevue (SAB), QC (45° 43′ N, 73° 94′ W) on a Chateauguay clay loam soil; Lévis (LEV), QC (46° 80′ N; 71° 09′ W) on a Kamouraska clay soil; and Normandin (NOR), QC (48° 50′ N, 72° 32′ W) on a Labarre clay loam soil. Two series of plots were seeded at each site with one to be sampled in 2015 and the other in 2016. Each series of plots was established with eight treatments consisting of mixtures of alfalfa with tall fescue or timothy seeded in the following proportions: 80:20, 60:40, 40:60, and 20:80. Plots were randomly assigned to a randomized complete block design with two replications. Each plot measured a minimum of 1.3 × 5 m; the exact size depending on the site. At each site, there was a total of 16 plots (8 treatments × 2 blocks) to be sampled in 2015 and 2016.

The alfalfa cultivar used in this experiment was ‘Calypso’, while the timothy and tall fescue cultivars were ‘AC Alliance’ and ‘Courtenay’, respectively. Seeding was done using a Fabro 7-row seeder (Swift Current, SK, Canada) at SAB and a Carter 6-row seeder (Brookston, IN) at LEV and NOR. Seeding rate varied by treatment and was based on weight of pure live seeds. Plots with alfalfa and timothy proportions of 80:20, 60:40, 40:60, and 20:80 were, respectively, seeded at the following rates: 12.8 and 3.2, 9.6 and 6.4, 6.4 and 9.6, and 3.2 and 12.8 kg ha^{-1} on a pure live seed basis. Plots with 80:20, 60:40, 40:60 and 20:80 of alfalfa and tall fescue were seeded at the following rates: 15.2 and 3.8, 11.4 and 7.6, 7.6 and 11.4, and 3.8 and

15.2 kg ha^{-1}, respectively, on a pure live seed basis. Fertilization was based on soil tests taken prior to seeding and following local recommendations (CRAAQ, 2010). All plots were harvested at the early flowering stage in 2014, the year preceding sampling, and in 2015 for the plots to be sampled in 2016.

3.1.2. Forage sampling

Each plot was sampled twice a week for four or five weeks (depending on alfalfa stage of development), starting when the alfalfa reached an average height of 40 cm. A 50 × 50 cm quadrat was thus used to collect eight to ten independent samples within each plot. A total of 832 forage samples were collected (416 in both 2015 and 2016).

Within quadrats, the data collected included: alfalfa maximum height (AMAXHT; length in cm of the tallest alfalfa stem from the ground to the terminal bud once fully extended), alfalfa maximum stage (AMAXSTAGE; stage of development of the most mature alfalfa stem) based on Kalu and Fick (1981), and grass maximum height (GMAXHT; length in cm of the tallest grass stem from the ground to the tip of the lastly emerged grass leaf). Samples were cut using scissors at a height of 7.5 cm and later separated by hand into grass, alfalfa, and weed components, bagged and dried at 55°C for 72 hours. The actual grass fraction was calculated based on the weight of the dried grass (GFRAC) against the weight of the total dried sample. The grass fraction group (GGRP) was defined as the 20% interval (i.e., 20, 40, 60 or 80 %) that was closest to the GFRAC value. The Julian date [JULIAN or DOY (Day of Year); number of days from the start of the year] at time of sampling was noted and data for the calculation of cumulated GDD was collected from weather stations located at each site. Growing degree days in °C were calculated for each experimental day using the following formula and then cumulated:

$$GDD = \left[\left(\frac{highest\ temp.in\ day + lowest\ temp.in\ day}{2}\right) - base\ temp.)\right]$$

The data collected and mean, range, and standard deviation for each variable determined are presented in Table 3.1.

<u>3.1.3. Chemical analyses</u>

After samples were dried at 55 °C, the alfalfa and grass fractions in each sample were recombined and ground using a Wiley mill (Standard model 4, Arthur H. Thomas Co., Swedesboro, NJ) to pass through a 1-mm screen. Ground forage samples from all sites were scanned by visible near infrared reflectance spectroscopy (VNIRS) using a NIRS DS2500 monochromator instrument (Foss NIRSystems Inc., Silver Spring, MD) with a small cup containing approximately 50 mL of forage sample. A calibration set of 48 samples in 2015 and 57 samples in 2016 were selected by the WinISI software version 4.5.0.1407 (Infrasoft International, LLC, Silver Spring, MD). These sets were chemically analyzed for the concentrations of acid detergent fibre (ADF), neutral detergent fibre analyzed using heat stable alpha (α)-amylase (aNDF), total nitrogen (TN) to be converted to crude protein (CP), ether extract (EE), *in vitro* true digestibility of DM (IVTD), and *in vitro* neutral detergent fibre digestibility (NDFd). Relative feed value (RFV) and relative feed quality (RFQ) were then calculated. The VNIRS prediction of each nutritive attribute was considered successful if 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) was greater than 3 (Nie et al., 2009).

The aNDF concentration was analyzed using heat stable α-amylase and sodium sulfite as per Van Soest et al. (1991) followed by ashing of the fibre residue to provide results corrected for the ash content of the fibre residue (aNDFom) (Mertens, 2011). The ADF concentration was determined using method 973.18 of the Association of Official Analytical Chemists (AOAC) (1990) followed by ashing of the fibre residue to provide results corrected for the ash content of the fibre residue (ADFom). The aNDFom and ADFom procedures were done with an Ankom200 Fibre Analyzer (Ankom Technology, Macedon, NY) using F57 filter bags. The EE determinations were performed using an Ankom XT15 Extractor (Ankom Technology, Macedon, NY) and XT4 filter bags following the AOCS procedure method AM 5-04 (AOCS, 2003). To determine TN concentration, a modified version of Isaac and Johnson (1976) protocol was used to extract nitrogen from plant material, and the 13-107-06-2-E method (Lachat Instruments, 2011) and an autoanalyser (QuikChem 8000 Lachat Zellweger Analytics, Inc., Lachat Instruments, Milwaukee, WI) was then used to measure TN. The IVTDom and NDFdom were determined using a 48-h incubation with buffered rumen fluid followed by an aNDF determination of the post-digestion residues (Goering and Van Soest, 1970), this was then followed by ashing of the fibre residue to provide results corrected for the ash content of the fibre residue. The rumen fluid incubation was performed by following the Ankom protocol utilizing bath incubation along with the Ankom Daisy II incubator and Ankom F57 filter (Ankom Technology, NY, USA). Rumen fluid was collected from a fistulated dairy cow. Finally, samples were analyzed for dry matter (DM) and ash concentration (Leco corporation, 2009) using a thermogravimetric analyser (model TGA701, Leco Corporation, St. Joseph, MI). Crude protein (CP) concentration was estimated as follows: CP = TN × 6.25. Values for relative feed value (RFV), and relative feed quality (RFQ) were calculated using the Excel spreadsheet Milk2013 (Undersander, 2013).

3.1.4. Statistical methods

The REG procedure of SAS (SAS version 9.4, SAS Institute, Cary, NC) was used to compute the results and statistical variables of interest. To ensure that only alfalfa-grass mixed samples were used, all samples with ≥ 10 % grass or ≤ 90 % grass were selected, resulting in a total of 679 samples. Data was inputted into the different equations from Parsons et al. (2006b, 2013) (Table 3.2) and the predicted results obtained using each equation were compared to the nutritive attribute values determined in the laboratory using VNIRS. The equations developed by Parsons et al. (2006b) provide results on g kg^{-1} DM basis; however, equations developed by Parsons et al. (2013) provide results on a % DM basis. To ensure consistency all results herein are reported on a g kg^{-1} DM basis. All equations tested were developed by Parsons et al. (2006b and 2013) using variables measured using the metric system, however, a correction (Parsons et al., 2014) provides the equations of Parsons et al. (2013) as originally intended with variables measured in the imperial system with the appropriately modified coefficients, these updated equations were not evaluated herein.

The equations were validated by regressing observed (VNRIS determined) values on the corresponding values predicted by the various equations developed by Parsons et al. (2006b and 2013) using the REG procedure in SAS (SAS version 9.4, SAS Institute, Cary, NC). The slope (*b*) and intercept (*a*) of all regression lines were determined and tested for the hypothesis that the slope was not significantly different from 1 and the intercept was not significantly different from 0. A prefect prediction equation would have a slope of 1 and an intercept of 0. The coefficient of determination (r^2) between observed and predicted values was reported along with the root mean square error (RMSE), the standard deviation about the regression line, with lower values being preferred when comparing predictive equations. The use of r^2 as a determining factor is due to its nature of providing understanding to what degree the equation variables explain the dependent variable results (Myers, 1990).

Table 3.1. Descriptive statistics for mixed alfalfa-grass forage samples collected at three sites in Quebec used to evaluate predictive equations for forage nutritive attributes developed in New York (Parsons et al., 2006b and 2013).

Trait	Units	Mean	Maximum	Minimum	SD
AMAXHT	cm	71	127	31	18.2
GMAXHT	cm	79	121	34	19.3
AMAXSTAGE	Scale	3.76	7	2	1.29
GFRAC	Decimal	0.39	0.89	0.10	0.19
GGRP	Decimal	0.40	0.80	0.20	0.19
DOY	days	162	187	135	12.2
GDD0	°C	669	954	376	154
GDD5	°C	369	589	181	103

AMAXHT, alfalfa maximum height (cm); GMAXHT, grass maximum height (cm); AMAXSTAGE, alfalfa maximum staged based on Kalu and Fick (1981); DOY, day of year; GDD0, growing degree days base 0°C; GDD5, growing degree days base 5°C; AFRAC, alfalfa fraction of sample written as a decimal; GFRAC, grass fraction of sample written as a decimal (e.g., 0.1 or 0.6); and GGRP, grass fraction group written as a decimal (e.g.., possible values are 0.2, 0.4, 0.6, and 0.8 with actual values assigned to the nearest value, for example 0.16 is assigned to 0.20).

Table 3.2. Equations developed by Parsons et al. (2013) to predict neutral detergent fibre (aNDF, % DM), acid detergent fibre (ADF, % DM), relative feed value (RFV), *in vitro* neutral detergent fibre digestibility based on 48-hr incubation (NDFd, % aNDF) and relative feed quality (RFQ) of alfalfa-grass mixtures that were evaluated for use in the Province of Québec.

Nutritive variable	Equations
aNDF	EQ6: 12.5 + (22.4 × GFRAC) + (0.315 × AMAXHT) EQ7:14.4 + (18.2 × GFRAC) + (0.238 × GMAXHT) EQ8: 9.79 + (23.2 × GFRAC) + (0.285 × AMAXHT) + (1.76 × AMAXSTAGE) EQ9: 6.28 + (24.5 × GFRAC) + (0.0425 × GDD0) EQ10: 4.89 + (24.7 × GFRAC) + (0.0241 × GDD0) + (0.198 × AMAXHT)
ADF	EQ11: 10.4 + (7.12 × GFRAC) + (0.254 × AMAXHT) EQ12: 11.9 + (3.70 × GFRAC) + (0.192 × GMAXHT) EQ13: 8.53 + (7.63 × GFRAC) + (0.233 × AMAXHT) + (1.21 × AMAXSTAGE) EQ14: 6.54 + (8.46 × GFRAC) + (0.0324 × GDD0) EQ15: 5.30 + (8.66 × GFRAC) + (0.0161 × GDD0) + (0.176 × AMAXHT)
NDFd	EQ16: 61.5 + (14.3 × GFRAC) – (0.204 × AMAXHT) EQ17: 60.8 + (17.0 × GFRAC) – (0.16 × GMAXHT) EQ18: 66.8 + (12.9 × GFRAC) – (0.146 × AMAXHT) – (3.37 × AMAXSTAGE) EQ19: 71.0 + (11.4 × GFRAC) – (0.0360 × GGD0) EQ20: 71.4 – (11.4 × GFRAC) – (0.0311 × GDD0) – (0.0524 × AMAXHT)
RFV	EQ21: 312 – (97.1 × GFRAC) – (1.73 × AMAXHT) EQ22: 300 – (73.6 × GFRAC) – (1.29 × GMAXHT) EQ23: 325 – (101 × GFRAC) – (1.59 × AMAXHT) – (8.38 × AMAXSTAGE) EQ24: 347 – (109 × GFRAC) – (0.234 × GDD0) EQ25: 354 – (110 × GFRAC) – (0.133 × GDD0) – (1.09 × AMAXHT)
RFQ	EQ26: 353 – (72.1 × GFRAC) – (2.21 × AMAXHT) EQ27: 339 – (42.3 × GFRAC) – (1.66 × GMAXHT) EQ28: 377 – (78.6 × GFRAC) – (1.95 × AMAXHT) – (15.2 × AMAXSTAGE) EQ29: 411 – (90.5 × GFRAC) – (0.320 × GDD0) EQ30: 420 – (91.8 × GFRAC) – (0.209 × GDD0) – (1.19 × AMAXHT)

AMAXHT, alfalfa maximum height (cm); GMAXHT, grass maximum height (cm); AMAXSTAGE, alfalfa maximum stage of development, Kalu and Fick (1981); GDD0, growing degree days base 0°C; GFRAC, grass fraction of sample, written as a decimal

3.2. Objective 2. Development of predictive equations of forage nutritive attributes for multiple growth cycles of mixed alfalfa-grass stands.

3.2.1. Experimental design and setup

Alfalfa and grass mixtures were seeded in May 2014 in Sainte-Anne-de-Bellevue (SAB), QC (45^0 43′ N, 73^0 94′ W), Lévis (LEV), QC (46^0 80′ N; 71^0 09′ W), and Normandin (NOR), QC (48^0 50′ N, 72^0 32′ W). Soil characteristics at each site were previously reported in section 3.1.1. At each site, two fields were seeded with one being sampled in 2015 and the other in 2016. Pure alfalfa and mixtures of alfalfa with tall fescue or timothy in the following ratios 80:20, 60:40, 40:60, and 20:80 were seeded, resulting in 8 treatments. Each treatment was established twice at each site with one plot being sampled during spring growth and the other during the first summer regrowth. Plots were randomly assigned to a randomized complete block design with split-plot restriction and two replications. Main plots were assigned to either spring growth or first summer regrowth, while sub-plots were assigned to the alfalfa-grass proportions treatments. Each sub-plot measured a minimum of 1.3 × 5 m; the exact size depending on the site.

Cultivars used in this experiment included ‘Calypso’, ‘AC Alliance’, and ‘Courtenay’ for alfalfa, timothy, and tall fescue, respectively. Seeding and plot management procedures were previously described in details in section 3.1.1. In summary, plots with 80:20, 60:40, 40:60 and 20:80 of alfalfa and a grass were seeded at the following rates (based on a pure live seed basis). For timothy and alfalfa: 12.8 and 3.2, 9.6 and 6.4, 6.4 and 9.6, and 3.2 and 12.8 kg ha^{-1}, respectively, while for tall fescue and alfalfa, it was: 15.2 and 3.8, 11.4 and 7.6, 7.6 and 11.4, and 3.8 and 15.2 kg ha^{-1}, respectively.

3.2.2. Field sampling

A total of 1798 samples were collected during the course of experimentation (800 in 2015 and 798 in 2016). Samples were taken from 50 × 50 cm quadrats. Once the alfalfa reached an average height of 40 cm during the initial growth, samples were taken twice a week for four or five weeks, resulting in 8 to 10 independent samples taken from different quadrats in each plot. The plot series sampled during the spring growth was then discarded. The plot series to be sampled during the first summer regrowth was cut at the same date during the spring growth when alfalfa reached the early flowering stage of development. These plot series was then allowed to regrow to an average alfalfa height of 30 cm before sampling of the regrowth was initiated. Samples taken during the initial spring growth occurred in different blocks then samples taken during the first summer regrowth of 2015; a second set of plot series was used for sampling in 2016.

Data collection was described in details in section 3.1.2. and included: alfalfa maximum height (AMAXHT; length in cm of the tallest alfalfa stem from the ground to the terminal bud once fully extended), alfalfa maximum stage (AMAXSTAGE; stage of development of the most mature alfalfa stem) based on Kalu and Fick (1981), grass maximum height (GMAXHT; length in cm of the tallest grass stem from the ground to the tip of the grass leaf), and grass maximum stage (GMAXSTAGE; stage of development of the most mature grass tiller) based on Moore et al. (1995). Samples were hand separated into alfalfa, grass, and weeds, and dried at 55°C for 72 hours to determine the actual grass and alfalfa contributions to total biomass on a dry matter basis (GFRAC and AFRAC). The grass fraction group (GGRP) was defined as the 20% interval that is closest to the GFRAC. The Julian date (JULIAN or DOY, day of the year; number of days from the start of the year) at time of sampling was determined along with the growing degree days (GDD) accumulated using a base temperature of 0°C (GDD0) and 5°C (GDD5).

3.2.3. Chemical analyses

Sample preparation, details of laboratory analyses and details of appropriate calculations were provided in section 3.1.3. In summary, visible near infrared reflectance spectroscopy (VNIRS) was used to predict all samples after a calibration set of samples was identified and chemically analyzed. A calibration set of 170 samples were selected by the WinISI software version 4.5.0.1407 (Infrasoft International, LLC, Silver Spring, MD). The set was chemically analyzed for the concentrations of acid detergent fibre corrected for the ash content of the fibre residue (ADFom), neutral detergent fibre analyzed using heat stable α-amylase corrected for the ash content of the fibre residue (aNDFom), total nitrogen (TN) to be converted to crude protein (CP), ether extract (EE), *in vitro* true digestibility of DM corrected for the ash content of the residue (IVTDom), and *in vitro* neutral detergent fibre digestibility corrected for the ash content of the residue (NDFdom). Calculations were also performed to determine the relative feed value (RFV) and relative feed quality. Results from chemical analysis and calculations were entered into a NIRS DS2500 monochromator instrument (Foss NIRSystems Inc., Silver Spring, MD) to predict the remaining samples.

3.2.4. Statistical methods

The complete plot data set was used to create predictive equations for nutritive attributes of interest for both the initial spring growth and the first summer regrowth of alfalfa grass mixtures. To ensure that only mixed alfalfa grass samples were used, all samples with less than 10% or greater than 90% grass were, however, removed, leaving a total of 1156 samples. The sample data was than analysed using the REG procedure in SAS (SAS version 9.4, SAS Institute, Cary, NC), with predictive equations being identified using the RSQUARE method. The three best two-, three-, and four-variable predictive equations were selected for each forage nutritive attribute of interest based on their R^2 with a targeted minimum ≥ 0.75. The statistical tools and the calculations used to evaluate the predictive equations included the coefficient of determination (R^2) between the predicted values and the values observed through laboratory analysis, the root mean square error (RMSE), the normalized RMSE (NRMSE), the prediction sum of squares (PRESS) and the mean 95% confidence interval (CI). The use of R^2 as a determining factor is due to its nature of providing understanding to what degree the equation variables explain the dependent variable results (Myers, 1990).

The RMSE is an estimator of the average difference between observed and predicted attributes, or the calibration error, with lower values being preferred when comparing predictive equations. The NRMSE is a standardized version of the root mean square error, an indicator of calibration error, which is corrected based on y_{max} - y_{min}, with y being the simulated value, and reported as a percentage. It can be used to rank predictive equations with a lower NRMSE being preferred.

$$\text{NRMSE (\%)} = \left(\frac{\text{RMSE}}{y_{max} - y_{min}} \right) \text{ x } 100$$

The PRESS variable is used as a predictive validation criterion. It is calculated by removing one observation at a time, predicting the coefficients for the predictive equations with the remaining observations, predicting the result of the removed observation and then replacing the initially removed observation before moving on to do the same process for the remaining data points. The residuals for each step of this process are summed with the total value being the

PRESS. When comparing predictive equations, the lower the PRESS statistic the better (Myers, 1990).

The mean 95% confidence interval (CI) is another tool used when creating statistical models as it indicates the upper and lower bounds for which one would find the prediction to fall within 95% of the time.

3.3. Objective 3. Evaluating the use of predictive equations of forage nutritive attributes on commercial farms across Québec.

3.3.1. Fields description

Samples were taken from producers' fields in twelve administrative regions of the province of Québec (Estrie, Laurentides, Abitibi-Témiscamingue, Centre-du-Québec, Montérégie-Ouest, Bas-Saint-Laurent, Gaspésie-Îles-de-la-Madeleine, Capitale-Nationale, Chaudière-Appalaches, Lanaudière, Outaouais, and Mauricie). In 2015, a total of 10 fields were sampled, while 13 fields were sampled in 2016. Fields selected were predominantly mixtures of alfalfa with either timothy or tall fescue. Fields varied in terms of soil characteristics, seeding, fertilization, and harvest management. Two protected areas of 8 m^2 were randomly selected in each field for data collection and sampling.

3.3.2. Field sampling

A total of 353 samples were collected from producers' fields (148 in 2015 and 205 in 2016). Plots were sampled twice a week for up to four weeks during the spring growth once the alfalfa reached an average height of 40 cm. Samples were taken within a 50 × 50 cm quadrat with each sample being taken from a previously unsampled portion of their respective 8 m^2 area, providing us with up to 8 independent samples per field.

As previously described in section 3.1.2., the data collected within the quadrats included: alfalfa maximum height (AMAXHT; length in cm of the tallest alfalfa stem from the ground to the terminal bud once fully extended), alfalfa maximum stage (AMAXSTAGE; stage of development of the most mature alfalfa stem) based on Kalu and Fick (1981), grass maximum height (GMAXHT; length in cm of the tallest grass stem from the ground to the tip of the grass leaf), grass maximum stage (GMAXSTAGE; stage of development of the most mature grass tiller) based on Moore et al. (1995), grass fraction (GFRAC; represented the contribution of grass to the total biomass and was determined visually), alfalfa fraction (AFRAC, i.e., alfalfa contribution to total biomass determined by subtracting GFRAC from 100% assuming that samples were weed-free), and grass fraction group (GGRP, defined as the 20% interval closest to the estimated GFRAC values , i.e., 20, 40, 60, and 80%). 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 hours. The Julian date (JULIAN or DOY; day of the year or number of days from the start of the year) at time of sampling was determined along with the accumulated growing degree days (GDD) using a base temperature of 0°C (GDD0) and 5°C (GDD5). Data for the calculation of GDD was collected from the nearest weather station to the producer field that could be accessed through the Environment Canada meteorological portal (Environment Canada, 2016). Growing degree days in °C were calculated using the following formula:

$$GDD = \left[\left(\frac{highest\ temp.in\ day + lowest\ temp.in\ day}{2}\right) - base\ temp.)\right]$$

3.3.3. Chemical analyses

All dried samples were ground using a Wiley mill (Standard model 4, Arthur H. Thomas Co., Swedesboro, NJ) to 1 mm for chemical analysis. A calibration set of 43 samples (22 samples in 2015 and 21 samples in 2016), selected by the WinISI software version 4.5.0.1407 (Infrasoft International, LLC, Silver Spring, MD), underwent chemical analysis with the results input into visible near infrared reflectance spectroscopy (VNIRS) to predict nutritive attributes in all samples. Details on the laboratory analysis procedures were previously detailed in section 3.1.3. In summary, the following analyses were performed on the calibration sets: the concentrations of acid detergent fibre corrected for the ash content of the fibre residue (ADFom), neutral detergent fibre analyzed using heat stable α-amylase and corrected for the ash content of the fibre residue (aNDFom), total nitrogen (TN) for conversion by calculation to crude protein (CP), ether extract (EE), *in vitro* true digestibility of DM corrected for the ash content of the residue (IVTDom), and *in vitro* neutral detergent fibre digestibility corrected for the ash content of the fibre residue (NDFdom). Calculations were then performed for relative feed value (RFV) and relative feed quality (RFQ). Results of the chemical analysis and calculations were entered into the WinISI software. The VNIRS prediction of each nutritive attribute was considered successful if 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) was greater than 3 (Nie et al., 2009).

3.3.4. Statistical methods

Statistical analysis was performed using the REG procedure in SAS (SAS version 9.4, SAS Institute, Cary, NC). Data was input into predictive equations previously developed in Québec in section 3.2. Those equations chosen for testing were the best two-, three-, and four-variable predictive equations based on statistical results and ease of use (Table 4.1). Details and procedures used for statistical analysis were previously described in section 3.1.4. In summary, the predicted results obtained from the equations were compared to the values determined by laboratory analysis. The mean observed and predicted values for each equation were reported as were the r^2 between the laboratory observed and predicted along with RMSE. Lastly, the equation of the trendline describing the relation between the observed and predicted values was also identified and the slope and intercept were tested to see if they were significantly different from the ideal (slope=1 and intercept=0) with the standard error for both the slope and intercept identified.

4. Results and Discussion

4.1. Objective 1. Evaluating the potential of predictive equations of forage nutritive attributes developed in New York State for use in Québec.

The predictive equations developed by Parsons et al. (2006b, 2013, 2014) in New York State, which will later be collectively referred to as NYPEAQ, were evaluated for their accuracy in predicting some nutritive attributes of alfalfa-grass mixtures grown in Québec using samples gathered from three locations (i.e., SAB, LEV, and NOR). To test the capabilities of the NYPEAQ equations, only mixed alfalfa and grass samples gathered during the initial spring growth were used. The reason for this was to follow as closely as possible the approach used to develop the NYPEAQ predictive equations that only predict nutritive attributes of first cut alfalfa-grass mixtures (Parsons et al., 2006b, 2013). The nutritive attributes to be predicted have been corrected for the ash content of the fibre residue, however, it is unclear whether the NYPEAQ equations were created to predict nutritive attributes with values that have undergone this correction. This correction was applied in our experiment as it is now the standard procedure used locally in Québec by many laboratories.

4.1.1. Neutral detergent fibre concentration corrected for the ash content of the fibre residue (aNDFom)

Overall the observed aNDFom values of the samples used for the evaluation of NYPEAQ predictive equations ranged between 264 and 615 g kg^{-1} DM averaging 437 g kg^{-1}, while the average predicted values when using the different NYPEAQ equations to predict forage aNDFom concentrations with data from Québec ranged between 402 and 478 g kg^{-1} DM depending on the equation used (Table 4.1). The absolute difference between mean observed and predicted values ranged from 1.2 to 41.8 g kg^{-1} DM. The coefficient of determination values (r^2) derived from the regressions of observed on predicted values ranged from 0.71 to 0.81, while the RMSE varied between 29.1 and 35.6 g kg^{-1}. All equations tested had significant bias with both the slope and the intercept being significantly different from 1 and 0, respectively. Such bias is not unexpected given the large number of samples that were included in the present study (i.e., n=679) and the relatively low RMSE we observed. Variation within both our slope coefficient and intercept was large and ranged between 0.69 and 0.99 and 36.5 and 118.3 g kg^{-1} DM, respectively, depending on the equation tested.

At the onset of experimentation we initially established that r^2 greater than 0.75 between laboratory observed and NYPEAQ-predicted values would be the minimum acceptable to determine that the NYPEAQ equations could be used in Québec. Based on the criteria initially laid out, four of the five equations developed by Parsons et al. (2006b) met or were above this minimum threshold of 0.75, while three of the five equations developed by Parsons et al. (2013) and evaluated in Québec were above this threshold.

Based on r^2 values, the best predictive equation for forage aNDFom concentration was equation 2 [$aNDFom = 91.2 + (2.1 \times AMAXHT) + (290 \times GFRAC) + (0.28 \times GDD5)$], which had an r^2 value of 0.81 (Table 4.1). This equation also had the best RMSE (i.e., the lowest value, 29.1 g kg^{-1} DM), which is a statistic that quantifies the predictive power of equations. The difference in mean observed and predicted values using equation 2 was although of 19 g kg^{-1} and this predictive equation had a slope (b = 0.76) and an intercept (a = 92) that were significantly different from 1 and 0, respectively. Based on these statistics, equation 2 was a strong predictive equation as it has the highest r^2; however, the accuracy of its predictions was not the best based on its slope, intercept, and difference between observed and predicted values compared to other equations tested. When predictions are made using this equation, it becomes less accurate as the forage matures and the aNDFom concentration increases (Figure 4.1a). This is due to the biased nature of the results, as seen by the slope and the intercept being significantly different from 1 and 0, respectively.

Another promising equation was equation 7. It had the third largest coefficient of determination with a value of 0.77, its RMSE was of 32.3 g kg^{-1} DM, and it had the largest slope coefficient (*b*) and the smallest intercept coefficient (*a*) with values of 0.99 and 36.5 respectively (Table 4.1). Both coefficients were significantly different from 1 and 0, respectively, again most likely due to the large number of samples we used (i.e., n= 679). The absolute difference in mean observed and predicted values was, however, of 35 g kg^{-1}.This equation consistently over predicted values across the range of observed aNDFom values (Figure 4.1b).

Finally, equations 1 and 6, despite having lower coefficient of determination (i.e., 0.75 and 0.74) and higher RMSE (i.e., 33.1 and 34.3 g kg^{-1} DM) than equations 2 and 7, had the lowest differences with average observed values, the differences being of 1.2 and 1.4 g kg^{-1} DM for equation 1 and 6, respectively. Equation 6 also had the third lowest RMSE, the second highest slope coefficient and the second lowest intercept. It had one of the lowest bias in predicting average aNDFom values, when compared to other equations evaluated (Figure 4.1c). In the case of equation 1 it had the third highest slope coefficient and an average intercept coefficient compared to other equations (Figure 4.1d).

Some of the statistics observed when testing the NYPEAQ equations to predict aNDFom with mixed alfalfa-grass samples collected in Québec are comparable to some values reported when other predictive equation of forage quality were tested in a range of environments. In the case of Parsons et al. (2006b) for equations developed to predict NDF of mixed alfalfa-grass samples the r^2 ranged between 0.85 and 0.95 and the RMSE between 19.5 and 34.2 g.kg^{-1} DM depending on the equation, parameter it included, and dataset used to test equations. In this particular case the samples were all collected in the State of New York. To our knowledge the equations developed by Parsons et al. (2006b, 2013) for predicting aNDF concentrations were not evaluated in regions other than New York State. Our validation results, however, are comparable to those reported by others that validated the use of aNDF predictive equation for pure alfalfa in multiple regions. For example, when comparing our results to those of Sulc et al. (1997) evaluating the use of the original PEAQ equation (Hintz and Albrecht, 1991) to predict the aNDF concentration of pure alfalfa in multiple US states, our r^2 values were similar with the exception of the results from New York State, which had a higher r^2 than that observed with our data, while our RMSE values were all higher. In the Czech Republic, Hakl et al. (2010) also evaluated the use of the PEAQ equation (Hintz and Albrecht, 1991) as well as their own equations, consisting of variables including some from PEAQ as well as GDD base 5°C. They reported an r^2 of 0.62 and 0.64 for the PEAQ equation and the equation combining PEAQ with GDD, which are both lower than our results. Lastly, we compared our results to those reported by Andrzejewska et al. (2014) in Poland, whom also tested the PEAQ equation (Hintz and Albrecht, 1991) as well as a one variable equation based on alfalfa maximum height (Parsons et al., 2006a). The reported r^2 of 0.92 and 0.88, respectively, were higher than the values we observed. The RMSE values reported were 16.2 g kg^{-1} DM when using PEAQ while it was 19.5 g kg^{-1} DM for Parsons et al. (2006a) equation; both values are lower than those we observed.

4.1.2. Acid detergent fibre concentration corrected for the ash content of the fibre residue (ADFom)

Observed ADFom values ranged from 204.5 to 408.6 g kg^{-1} DM, with a mean observed value of 314 g kg^{-1} DM, while the range of predicted values using equations developed by Parsons et al. (2013), ranged from 135 to 467 g kg^{-1} DM, with the mean observed values ranging from 285 to 326 g kg^{-1} DM depending on the equation used (Table 4.1). The absolute difference between the mean observed and mean predicted varied between 1 and 29 g kg^{-1} DM. The r^2 values varied from 0.70 to 0.81, while the RMSE varied from 18.4 to 22.7 g kg^{-1} DM. The slope (b) and intercept (a) of the equations were determined and tested for difference from the ideal (b=1, a=0), as was done in the case of aNDFom. The results for the slope ranged from 0.68 to 0.91 and the intercept ranged from 53.6 to 100.8 g kg^{-1} DM depending on the equations tested, with all results being significantly different from the ideal.

As mentioned earlier, we initially identified a minimum threshold of $r^2 \geq 0.75$ for the predictive equations of ADFom. Three of five equations evaluated had an r^2 higher than this value (Table 4.1). The best equation based on r^2 was equation 15 [$ADFom = 53.0 + (86.6 \times GFRAC) + (0.161 \times GDD0) + (1.76 \times AMAXHT)$] with an r^2 of 0.81. The slope was significantly different from 1 (b = 0.68; $P < 0.0001$) and the intercept was significantly different from 0 (a = 96.8; $P < 0.0001$). Despite having the highest r^2 of the equations tested for predicting ADFom concentration as well as having the lowest RMSE, equation 15 had one of the smallest slope value and the second larger intercept, both significantly different from the ideal, indicating bias. Equation 15 under predicts lower values and overpredicts higher ADFom concentration values. This bias can be seen by viewing the graphed results between the ADFom concentration observed values and those predicted by equation 15 (Figure 4.2a). The difference between the mean observed and predicted ADFom values was of 5 g kg^{-1} DM, which could be considered as being minimal.

The best predictive equation of ADFom concentration based on the slope and intercept that met our minimum threshold of $r^2 \geq 0.75$ was equation 11 [$ADFom = 104 + (71.2 \times GFRAC) + (2.54 \times AMAXHT)$], which had an r^2 of 0.75 (Table 4.1). The slope (b = 0.80; $P = < 0.0001$) and intercept (a = 65.81; $P = < 0.0001$) were both significantly different than the ideal values (a = 0; b = 1) but were closer than other ADFom predictive equations that met our minimum r^2 threshold (Figure 4.2b). Equation 11 had the third highest RMSE of those equations predicting ADFom that were evaluated, indicating increased error in its predictions compared to others. As with other predictive equations for ADFom concentration the results were biased as the slope and intercept were significantly different from the ideal, however, equation 11 was the least biased when compared to other equations evaluated. The difference between the mean observed and predicted ADFom values was only 2 g kg^{-1} DM.

Comparing our results to the literature was again difficult as in the case of aNDFom concentration, the predictive equations developed prior to those of Parsons et al. (2006b, 2013) were to predict ADF concentration of pure alfalfa fields. When comparing our results to those reported by Fick and Janson (1990) for pure alfalfa, our observed r^2 were similar to those reported for two equations based on the Mean Stage Weight (i.e., New York MSW and national MSW equations), which had r^2 of 0.74 and 0.78, respectively, but were lower than what was reported for the best national equation, which was 0.94. For RMSE, the result reported for the New York MSW equation (Kalu and Fick, 1981) was 23.8 g kg^{-1} DM, which was comparable to the range we observed using Parsons et al. (2013) ADF equations (i.e., 18.4 to 22.7 g kg^{-1} DM). For three of the five NYPEAQ equations we evaluated the resulting RMSE values were lower than what was reported for the national MSW equation (i.e., 21.8 g kg^{-1} DM) by Fick and Janson

(1990), and our observed results for all equations evaluated were higher than the RMSE value they reported for the best national equation (i.e., 11.3 g kg^{-1} DM).

Comparing our results to those reported of Sulc et al. (1997), whom evaluated the use of a PEAQ equation predicting ADF concentration of pure alfalfa stands, the r^2 values we observed were comparable to those reported when the PEAQ equation was used in multiple States in the USA, with the exception of the value for New York State, which was much higher (i.e., r^2 of 0.87). The RMSE values we observed were similar for equations 13 and 15 when compared to those reported in multiple States by Sulc et al. (1997), however, the remaining three equations all had larger values. In the Czech Republic, Hakl et al., (2010) reported an r^2 values of 0.92 when using the PEAQ equation (Hintz and Albrecht, 1991) with data from pure alfalfa fields taken between 2004 and 2007 and an r^2 of 0.94 when using an equation combining PEAQ variables and GDD based 5°C. This is a larger value than the r^2 result of 0.70-0.81 we observed for mixed alfalfa-grass stands when using NYPEAQ equations (Parsons et al., 2013) with data from Québec. In Poland, Andrzejewska et al. (2014) reported an r^2 of 0.92 when testing data from pure alfalfa fields taken from 2009-2011 using PEAQ (Hintz and Albrecht, 1991) and an RMSE of 12.8 g kg^{-1} DM; this r^2 value is larger while the RMSE value is lower than we observed. The overall better results reported in multiple environments for the use of predictive equation for pure alfalfa ADF concentrations compared to results we observed for the use of similar equations to predict ADFom concentration of mixed alfalfa-grass stands might be associated with the greater variation associated with mixed species samples compared to single species ones.

4.1.3. *In vitro* digestibility of the neutral detergent fibre corrected for the ash content of the residue (NDFdom)

Observed NDFdom values ranged from 391 to 888 g kg^{-1} aNDFom with a mean value of 665 g kg^{-1} aNDFom while the predicted values ranged from 321 to 679 g kg^{-1} aNDFom with a mean value ranging from 488 to 548 g kg^{-1} aNDFom depending on the predictive equation used (Table 4.1). The absolute difference between mean observed and mean predicted values ranged from 117 to 177 g kg^{-1} aNDFom, again depending on the predictive equation used. The r^2 values between predicted and observed ranged from 0.52 to 0.73, while the RMSE values ranged from 48.2 to 63.8 g kg^{-1} aNDFom. The slope ranged from 1.11 to 1.50, while the intercept ranged from -157 to 125 g kg^{-1} aNDFom with all equations evaluated having the slope and intercept being significantly different from the ideal (a=0, b=1).

The best equation for predicting NDFdom based on r^2 was equation 18 [$NDFdom = 668 + (129 \times GFRAC) - (1.46 \times AMAXHT) - (33.7 \times AMAXSTAGE)$] with an r^2 of 0.73 (Table 4.1). There was quite a large difference from the other equations evaluated, which had an r^2 ranging from 0.52 to 0.54. Equation 18 also had the lowest RMSE. It had a slope closest to 1 (b = 1.11; $P < 0.0001$), and an intercept closest to 0 (a = 124; $P < 0.0001$) compared to the other NDFdom predictive equations evaluated, but both slope and intercept were significantly different from the ideal indicating a bias in results. To our knowledge no other studies have previously evaluated the use of predictive equations of forage NDFdom with which we could compare our results. None of the equations developed by Parsons et al. (2013) for predicting NDFd met our *a priori* minimum threshold ($r^2 \geq 0.75$) and all had significant bias in predicted values, thus we would not recommend the use of these equations in Québec.

4.1.4. Relative feed value (RFV)

The observed RFV values varied from 61 to 209 units with a mean value of 132 while the predicted values varied from 39 to 269 units with the mean values ranging from 142 to 170 units. The absolute difference between the observed mean and predicted means ranged from 10 to 38 units depending on the predictive equation used (Table 4.1). The slope (b) and intercept (a) ranged from 0.58 to 0.83 and -8.5 to 49 units, respectively, with results from all equations being significantly from the ideal (b = 1, a = 0).

Three of five equations predicting RFV met our minimum threshold ($r^2 \geq 0.75$) (Table 4.1). The best equation based on r^2 was the equation 25 [$RFV = 354 - (110 \times GFRAC) - (0.133 \times GDD0) - (1.09 \times AMAXHT)$], which had an r^2 of 0.78. It also had the lowest RMSE at 12.9 units. However, the slope was the smallest (b = 0.58; $P < 0.0001$) and it had the largest intercept (a = 47.3; $P < 0.0001$) of all the RFV predictive equations evaluated, with both the slope and intercept being significantly different from the ideal, indicating bias in predicted results. Despite having the highest r^2, this equation produced biased results manifested by under predicting the observed RFV at the lowest extreme and over predicting it at almost all other points. The absolute difference between the mean observed and predicted values was of 13 units (Figure 4.3a).

The best RFV equation based on slope and intercept was equation 22 [$RFV = 300 - (73.6 \times GFRAC) - (1.29 \times GMAXHT)$], which had an r^2 of 0.75 and the third highest RMSE at 13.9 units (Table 4.1). The slope was significantly different from 1 (b = 0.83; $P < 0.0001$) and intercept was significantly different from 0 (a = -8.51; $P = 0.0072$). The RFV predictions using this equation have the largest difference (i.e., 38 units) between observed and predicted values of the equations evaluated for predicting RFV that met our minimum r^2 threshold, however, the results are the least biased based on the slope and the intercept values. Predictions made with this equation on average follow the "true" RFV values, but it will always significantly overpredict RFV. This important bias could limit the potential use of this equation in Quebec despite meeting our *a priori* minimum threshold ($r^2 \geq 0.75$). As with NDFdom, there are no other studies that have looked at predicting RFV through predictive equations with which we can draw comparisons to for our results predicting RFV (Figure 4.3b).

4.1.5. Relative feed quality (RFQ)

The observed RFQ values ranged from 49 to 238 units, with a mean observed value of 137 units, while the predicted values ranged from 25 to 321 units, with the mean predicted values ranging from 151 to 192 units depending on the predictive equation used (Table 4.1). The absolute difference between the mean observed and mean predicted ranged from 14 to 45 units. The r^2 values between observed and predicted RFQ ranged from 0.70 to 0.80 and the RMSE varied from 17.2 to 21.3 units. The slope varied from 0.64 to 0.97, with only equation 27 having a slope that was not significantly different from 1 (a=0.97, $P = 0.16$) while the intercept ranged from -48.6 to 35.5 units, with only equation 26 having an intercept that was not significantly different from 0 (a = -5.10, $P = 0.12$).

Three of the five equations predicting RFQ met our minimum r^2 threshold value. The best was equation 30 [$RFQ = 420 - (91.8 \times GFRAC) - (0.209 \times GDD0) - (1.19 \times AMAXHT)$] with an r^2 of 0.80 (Table 4.1). The RMSE associated with this equation was the lowest of those equations that met our r^2 threshold. The slope (b = 0.65; $P < 0.0001$) and intercept (a = 31.83; $P < 0.0001$) were significantly different from the ideal (b = 1; a = 0), indicating biased results. The bias had less of an impact at lower RFQ values, as the observed and predicted values are closer to each other; it became a greater concern for higher values as the equation will over predict the observed values to a greater degree as the RFQ value increases. The absolute difference between mean observed and predicted values using this equation was of 23 units (Figure 4.4a).

The best RFQ predictive equation based on slope and intercept, which met our minimum r^2 threshold, was equation 26 [$RFQ = 353 - (72.1 \times GFRAC) - (2.21 \times AMAXHT)$], which had an r^2 of 0.75 (Table 4.1). The slope (b = 0.84; $P < 0.0001$) is significantly different from the ideal (b = 0) and the intercept (a = -5.10; $P = 0.1184$) is not significantly different from the ideal (a = 0) but there is still bias in results. The predictions follow the trend of the real RFQ values despite over predicting at all points with the margin of over prediction increasing as RFQ values increase. The absolute difference between mean observed and predicted values using this equation was of 31 units (Figure 4.4b). As with NDFdom and RFV, there are no other studies that have looked at predicting RFQ through predictive equations with which we can draw comparisons to for our results predicting RFQ.

4.1.6. Practical Implications

Some of the equations developed by Parsons et al. (2006b, 2013) to predict forage quality attributes of alfalfa-grass mixtures during the spring growth appear to have potential for use in Québec and could become a useful tool to help them determine the optimal time to harvest their forage fields. However, of the equations that appear to have potential all had some limitations most notably a consistent bias for most attributes. For a given attribute, the choice of a specific predictive equation to be used in Québec depends on several factors including i) the purpose of using the equation and how accurate the prediction must be (i.e., production or research conditions), ii) the equation ease of use with respect to measured variables required to make the prediction, and iii) statistics associated with the equation during validation. The choice of using a specific decision will depends on the weight given to these three criteria.

In the case of aNDFom equations 1, 2, 6, and 7 are the most promising. If equations 2 and 7 had some of the best statistics in terms of r^2 and RMSE values, they also had some of the largest bias as demonstrated by low slope coefficient values and/or absolute differences between mean observed and predicted values (Table 4.1 and Figure 4.1). In the case of equation 2 it over predicts aNDFom concentrations as values increase, while in the case of equation 7 it under predicts values but this under prediction is consistent across the entire range of values included in our dataset. In the case of equations 1 and 6, their r^2 and RMSE values were not as desirable, but their bias was smaller especially for values around our mean observed aNDFom (i.e., 437 g kg^{-1}). The absolute differences between mean observed and predicted values for both equations was of only 2 g kg^{-1}. Finally, all of these four equations included comparable variables namely GFRAC and AMAXHT or GMAXHT, but only equation 2 included growing degree days, which could be more difficult to retrieve for some potential users. Thus considering the three criteria presented earlier equations 1, 6, and 7 appear to have the most potential.

Some of the NYPEAQ predictive equations for ADFom appeared to have potential for use in Québec. Equation 11 [ADFom = 104 + (71.2 × GFRAC) + (2.54 × AMAXHT)] appeared to be the strongest candidate for use in Québec as it is a relatively simple two-variable equation with overall the best predictive capabilities and the lowest bias of the equations evaluated to predict ADF concentration (Table 4.1 and Figure 4.2). Again potential users of this equation in Quebec should be aware of this bias in the results predicted when using this equations. For RFV, equations 22 [RFV = 300 – (73.6 × GFRAC) – (1.29 × GMAXHT)] and 25 [RFV = 354 – (110 × GFRAC) – (0.133 × GDD0) – (1.09 × AMAXHT)] were the most promising, however, both were associated with significant bias in predicted results. However, in the case of equation 22 overestimation of RFV values was consistent across a range of values and thus could be accounted for. In addition, equation 22 is simpler to use as it includes GFRAC and GMAXHT and no growing degree days variable (Table 4.1 and Figure 4.3). Finally for RFQ, two of the most promising equation for use in Quebec could be equations 30 [RFQ= 420-(91.8×GFRAC)-(0.209×GDD0)-(1.19×AMAXHT)] and 26 [$RFQ = 353 - (72.1 \times GFRAC) - (2.21 \times AMAXHT)$]. However, as mentioned for other predictive equations both were associated with a significant bias in predicted results (Table 4.1 and Figure 4.4).

The significant bias associated with most of the predictive equations we evaluated could be due to a range of factors. One could be associated with the greater range in maturity of the alfalfa plants sampled when compared to values reported by Parsons et al. (2006b and 2013). Indeed, they reported that alfalfa plants in their samples ranged between developmental stages of 1 and 4 using Kalu and Fick (1981) system. In our case, the plants we sampled ranged between stages 2 and 7 (Table 4.1). When examining the bias associated with the use of the aNDFom, ADFom, RFV and RFQ equations evaluated many of them tended to overestimate higher values, while all values for NDFdom were under estimated. Other possible reasons for this bias and tendency to overestimate higher values could also be associated with differences in the predominant grass species that were used. While Parsons et al. (2006b) reported using in part samples from experimental plots of alfalfa mixtures with timothy, orchardgrass (*Dactylis glomerata* L.), and reed canarygrass (*Phalaris arundinacea* L.), in our case we used alfalfa mixtures with tall fescue and timothy. Differences in growth patterns and development, which impact changes in accumulation of cellulose, hemicellulose and lignin components, might affect the evolution of forage quality attributes as swards matures. It would be expected that such differences increase with time (e.g., Cherney et al., 1993; Karn et al., 2006). Another possible source for this bias could be a difference in methodology as Parsons et al. (2006b, 2013) cut plots to a height of 10 cm, while in our case the cutting height was of 7.5 cm as that is often the local practice in Québec. The excess stem at the lower portion of the plant will have increased fibre content that may not be properly accounted for in Parsons et al. (2006b, 2013) models.

Table 4.1. Validation of the use of existing predictive equations (Parsons et al., 2006b and 2013) with data from mixed alfalfa-grass fields from Québec. The predictive equations were used to predict the following forage nutritive attributes: neutral detergent fibre corrected for the organic matter content of the residue (aNDFom, g kg^{-1} DM), acid detergent fibre corrected for the organic matter content of the residue (ADFom, g kg^{-1} DM), in vitro neutral detergent fibre digestibility based on 48-hr incubation and corrected for the organic matter content of the residue (NDFdom, g kg^{-1} aNDF), relative feed value (RFV) and relative feed quality (RFQ) (n= 679). The predictive equations for aNDFom, ADFom and NDFdom nutritive attributes from 2013 were modified to provide results in g kg^{-1} through modification of intercept and coefficients.

Equations (EQ)	Mean obs.	Mean pred.	r^2	RMSE	Slope coefficient (b)	SEb	Prob b = 1	Intercept coefficient (a)	SEa	Prob a = 0
<u>2006 equations</u>										
EQ1: aNDFom = 87.1 + (3.2 × AMAXHT) + (313 × GFRAC)	437	435	0.75	33.05	0.77	0.017	<0.0001	101.0	7.47	<0.0001
EQ2: aNDFom = 91.2 + (2.1 × AMAXHT) + (290 × GFRAC) + (0.28 × GDD5)	437	456	0.81	29.14	0.76	0.014	<0.0001	92.1	6.53	<0.0001
EQ3: aNDFom = -229 + (2.6 × AMAXHT) + (307 × GFRAC) + (2.5 × DOY)	437	478	0.76	32.50	0.69	0.0148	< 0.0001	105.93	7.20	<0.0001
EQ4: aNDFom = 95.6 + (3.4 × AMAXHT) + (292 × GGRP)	437	453	0.71	35.63	0.76	0.0183	<0.0001	94.33	8.43	< 0.0001
EQ5: aNDFom = 97.9 + (2.1 × AMAXHT) + (269 × GGRP) + (0.29 × GDD5)	437	461	0.77	32.04	0.75	0.0159	<0.0001	89.12	7.42	<0.0001
<u>2013 equations</u>										
EQ6: aNDFom = 125 + (224 × GFRAC) + (3.15 × AMAXHT)	437	435	0.74	34.27	0.89	0.020	< 0.0001	50.66	8.98	< 0.0001

EQ7: aNDFom = 144 + (182 × GFRAC) + (2.38 × GMAXHT)	437	402	0.77	32.25	0.99	0.021	< 0.0001	36.46	8.59	< 0.0001
EQ8: aNDFom = 97.9 + (232 × GFRAC) + (2.85 × AMAXHT) + (17.6 × AMAXSTAGE)	437	456	0.73	34.50	0.76	0.018	<0.0001	91.49	8.13	<0.0001
EQ9: aNDFom = 62.8 + (245 × GFRAC) + (0.425 × GDD0)	437	442	0.76	32.82	0.72	0.016	<0.0001	118.25	7.03	<0.0001
EQ10: aNDFom = 48.9 + (247 × GFRAC) + (0.241 × GDD0) + (1.98 × AMAXHT)	437	446	0.78	31.30	0.73	0.015	<0.0001	110.32	6.77	<0.0001
EQ11: ADFom = 104 + (71.2 × GFRAC) + (2.54 × AMAXHT)	314	312	0.75	20.70	0.80	0.018	<0.0001	65.81	5.51	<0.0001
EQ12: ADFom = 119 + (37.0 × GFRAC) + (1.92 × GMAXHT)	314	285	0.70	22.70	0.91	0.023	0.0002	53.63	6.55	<0.0001
EQ13: ADFom = 85.3 + (76.3 × GFRAC) + (2.33 × AMAXHT) + (12.1 × AMAXSTAGE)	314	326	0.79	19.12	0.69	0.014	<0.0001	89.88	4.50	<0.0001
EQ14: ADFom = 65.4 + (84.6 × GFRAC) + (0.324 × GDD0)	314	315	0.72	22.00	0.68	0.016	<0.0001	100.76	5.16	<0.0001
EQ15: ADFom = 53.0 + (86.6 × GFRAC) + (0.161 × GDD0) + (1.76 × AMAXHT)	314	319	0.81	18.37	0.68	0.013	<0.0001	96.76	4.16	<0.0001
EQ16: NDFdom = 615 + (143 × GFRAC) - (2.04 × AMAXHT)	665	526	0.54	62.71	1.33	0.047	<0.0001	-37.45	25.06	0.1356
EQ17: NDFdom = 608 + (170 × GFRAC) - (1.6 × GMAXHT)	665	548	0.54	62.51	1.50	0.053	<0.0001	-156.65	29.11	<0.0001
EQ18: NDFdom = 668 + (129 × GFRAC) - (1.46 × AMAXHT) - (33.7 × AMAXSTAGE)	665	488	0.73	48.19	1.11	0.026	<0.0001	124.51	12.83	<0.0001

EQ19: NDFdom = 710 + (114 × GFRAC) - (0.360 × GGD0)	665	514	0.52	63.78	1.12	0.041	<0.0001	88.87	21.26	0.0034
EQ20: NDFdom = 714 + (114 × GFRAC) - (0.311 × GDD0) - (0.524 × AMAXHT)	665	513	0.54	62.46	1.12	0.039	<0.0001	91.85	20.34	0.0033
EQ21: RFV = 312 – (97.1 × GFRAC) – (1.73 × AMAXHT)	132	152	0.71	14.89	0.71	0.017	<0.0001	24.88	2.67	<0.0001
EQ22: RFV = 300 – (73.6 × GFRAC) – (1.29 × GMAXHT)	132	170	0.75	13.89	0.83	0.018	<0.0001	-8.51	3.16	0.0072
EQ23: RFV = 325 – (101 × GFRAC) – (1.59 × AMAXHT) – (8.38 × AMAXSTAGE)	132	142	0.72	14.65	0.61	0.015	<0.0001	45.69	2.13	<0.0001
EQ24: RFV = 341 – (109 × GFRAC) – (0.234 × GDD0)	132	148	0.77	13.45	0.58	0.012	<0.0001	49.00	1.84	<0.0001
EQ25: RFV = 354 – (110 × GFRAC) – (0.133 × GDD0) – (1.09 × AMAXHT)	132	145	0.78	12.94	0.58	0.012	<0.0001	47.31	1.79	<0.0001
EQ26: RFQ = 353 – (72.1 × GFRAC) – (2.21 × AMAXHT)	137	168	0.75	19.52	0.84	0.019	<0.0001	-5.10	3.26	0.1184
EQ27: RFQ = 339 – (42.3 × GFRAC) – (1.66 × GMAXHT)	137	192	0.70	21.30	0.97	0.024	0.1591	-48.63	4.75	<0.0001
EQ28: RFQ = 377 – (78.6 × GFRAC) – (1.95 × AMAXHT) – (15.2 × AMAXSTAGE)	137	151	0.77	18.60	0.67	0.014	<0.0001	35.45	2.24	<0.0001
EQ29: RFQ = 411 – (90.5 × GFRAC) – (0.320 × GDD0)	137	162	0.74	19.91	0.64	0.015	<0.0001	33.35	2.50	<0.0001
EQ30: RFQ = 420 – (91.8 × GFRAC) – (0.209 × GDD0) – (1.19 × AMAXHT)	137	160	0.80	17.23	0.65	0.013	<0.0001	31.83	2.11	<0.0001

r^2: coefficient of determination; RMSE: root mean square error; AMAXHT, alfalfa maximum height (cm); GMAXHT, grass maximum height (cm); AMAXSTAGE, alfalfa maximum staged based on Kalu and Fick (1981); GDD0, growing degree days base 0°C; GDD5, growing degree days base 5°C; DOY, day of the year; GFRAC, grass fraction of sample written as a decimal; and GGRP, grass fraction group, with group defined as the 20% interval closest to the GFRAC, written as a decimal (i.e., 0.16, 0.20).

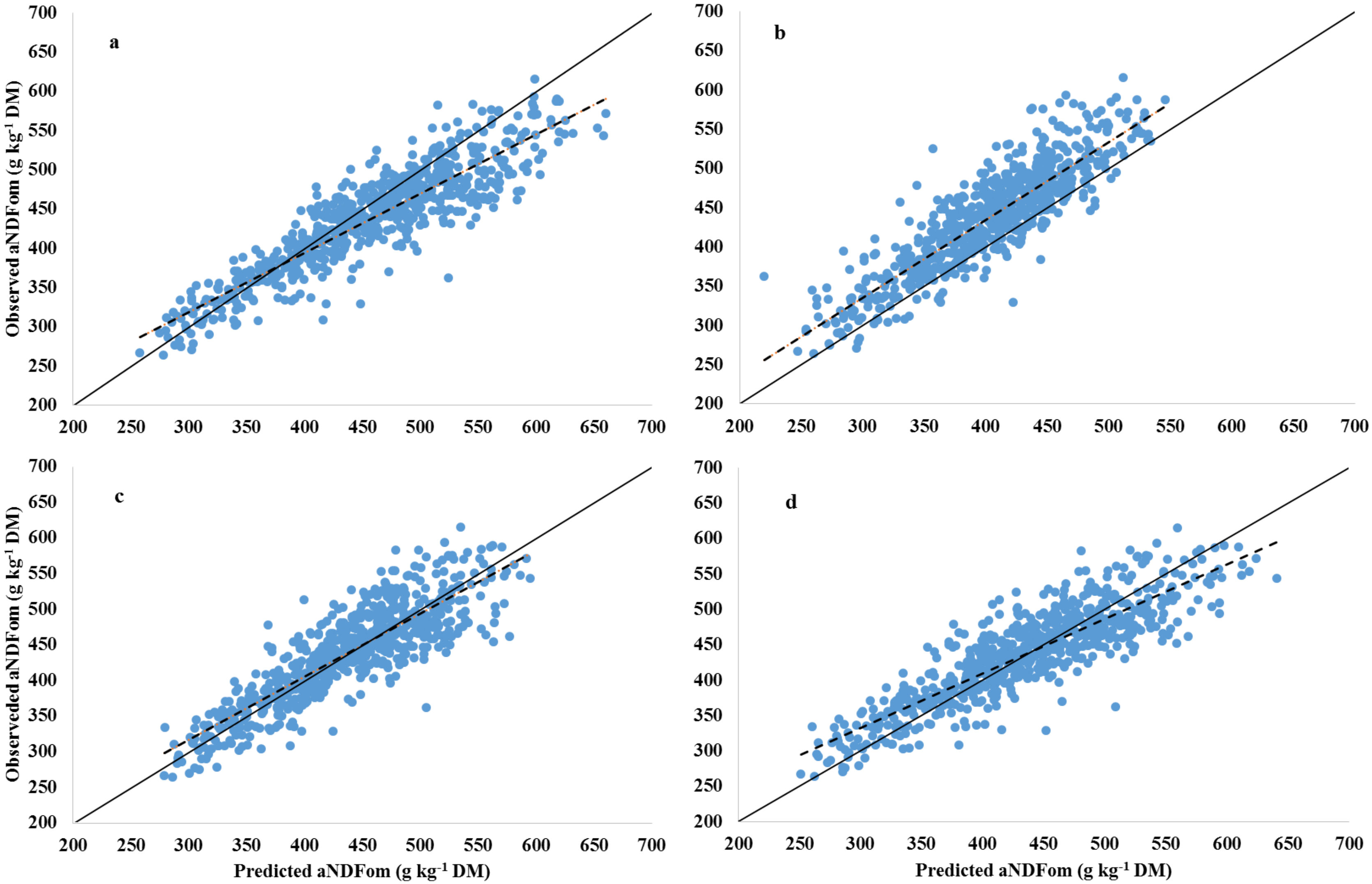

Figure 4.1 Relationship between forage neutral detergent fibre concentrations (determined using alpha amylase and corrected for the concentration of organic matter in the residue, aNDFom) laboratory observed values and those values predicted using predictive equations developed by Parsons et al. (2006b and 2013) in New York State of alfalfa-grass mixtures samples grown in Québec. a) equation 2, [aNDFom = 91.2 + (2.1 × AMAXHT) + (290 × GFRAC) + (0.28 × GDD5)]; b) equation 7 [aNDFom = 144 + (182 × GFRAC) + (2.38 × GMAXHT)]; c) equation 6 [aNDFom = 125 + (224 × GFRAC) + (3.15 × AMAXHT)], d) equation 1 [aNDFom = 87.1 + (3.2 × AMAXHT) + (313 × GFRAC)]. AMAXHT, alfalfa maximum height in centimeters; GFRAC, proportion of grass within samples based on the DM weight (e.g., 0.1 or 0.6); GDD5, growing degree days based 5°C; GMAXHT, grass maximum height in centimeters. The solid line indicates the ideal 1:1 relationship while the dotted line represents the regression line.

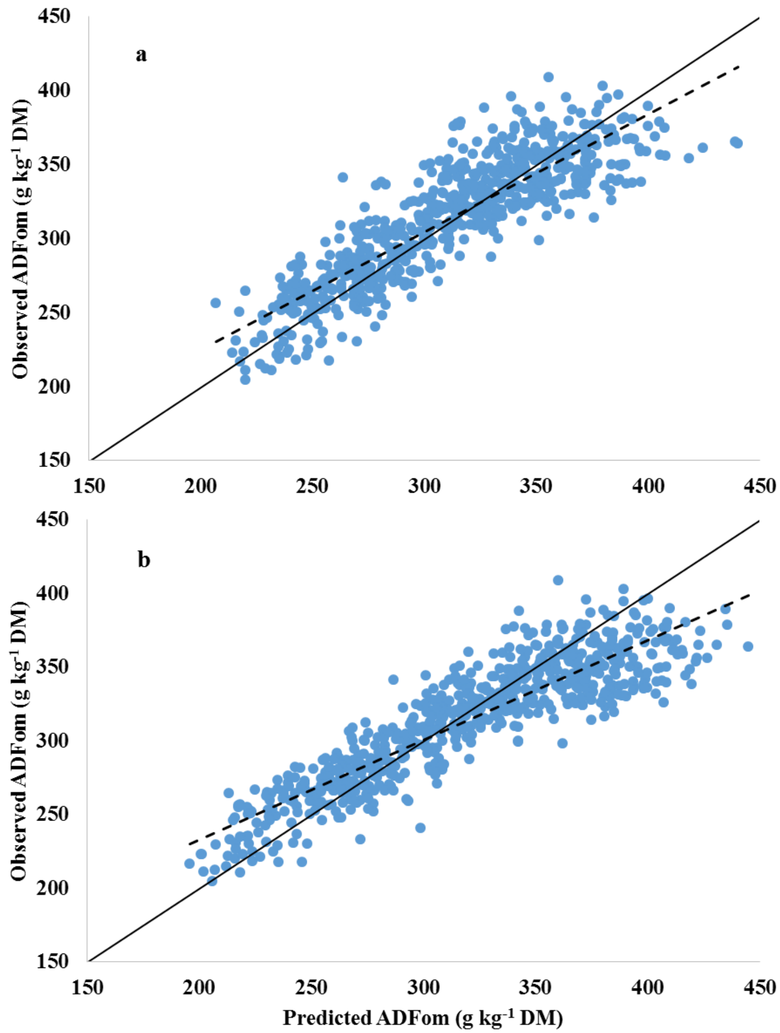

Figure 4.2 Relationship between forage acid detergent fibre concentrations (corrected for the concentration of organic matter in the residue, ADFom) observed values and those values predicted using predictive equations developed by Parsons et al. (2013) in New York State of alfalfa-grass mixtures samples grown in Québec. a) equation 15, [ADFom = 53 + (86.6 × GFRAC) + (0.161 × GDD0) + (1.76 × AMAXHT)]; b) equation 11 [ADFom = 104 + (71.2 × GFRAC) + (2.54 × AMAXHT)]. AMAXHT, alfalfa maximum height in centimeters; GFRAC, proportion of grass within samples based on the DM weight (e.g., 0.1 or 0.6); GDD0, growing degree days based 0°C. The solid line indicates the ideal 1:1 relationship while the dotted line represents the regression line.

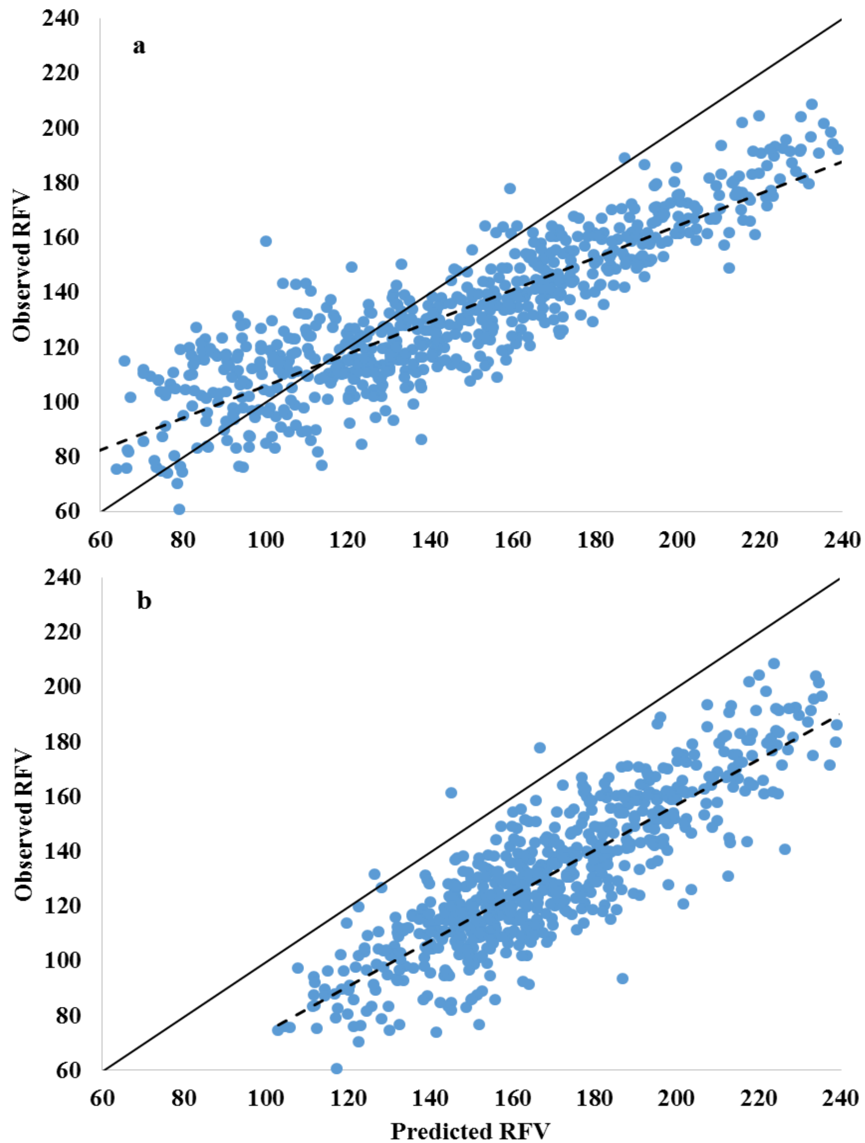

Figure 4.3 Relationship between Relative Feed Value (RFV) observed values and those values predicted using predictive equations developed by Parsons et al. (2013) in New York State of alfalfa-grass mixtures samples grown in Québec. a) equation 25, [RFV = 354 - (110 × GFRAC) + (0.133 × GDD0) - (1.09 × AMAXHT)]; b) equation 22 [RFV = 300 - (73.6 × GFRAC) - (1.29 × GMAXHT)]. AMAXHT, alfalfa maximum height in centimeters; GFRAC, proportion of grass within samples based on the DM weight (e.g., 0.1 or 0.6); GDD0, growing degree days based 0°C; GMAXHT, grass maximum height in centimeters. The solid line indicates the ideal 1:1 relationship while the dotted line represents the regression line.

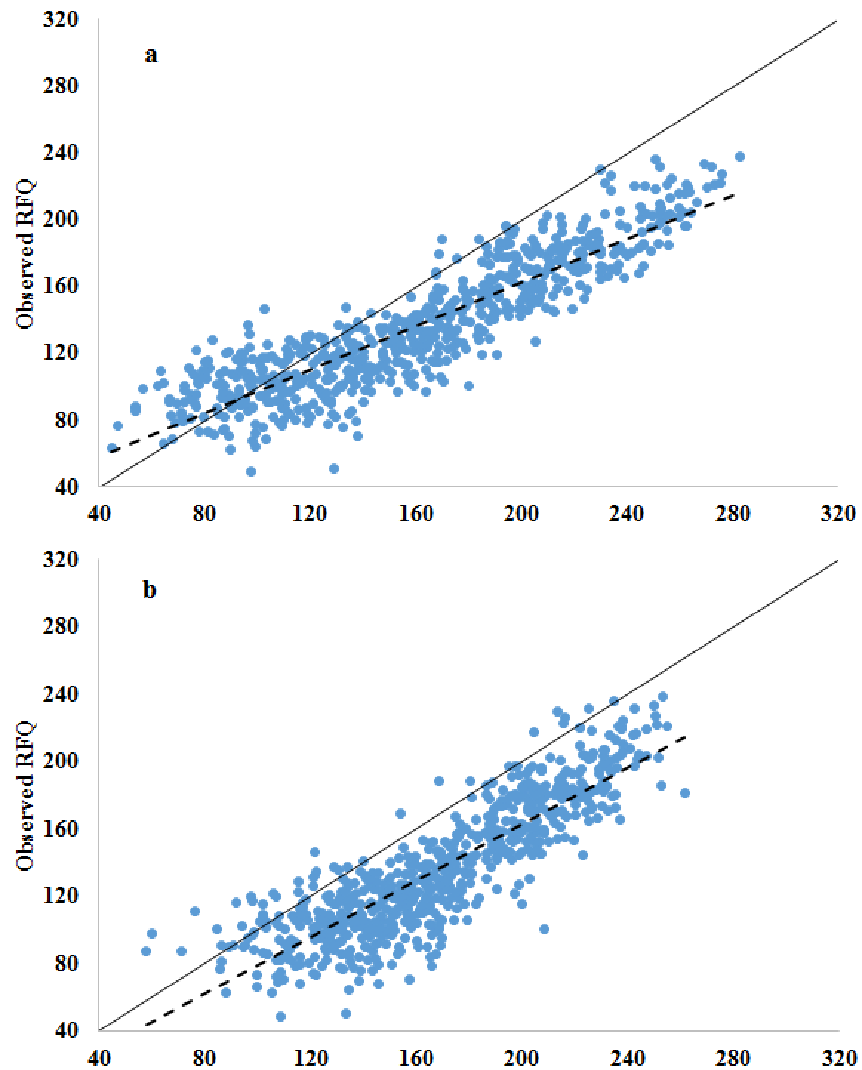

Figure 4.4 Relationship between Relative Feed Value (RFV) observed values and those values predicted using predictive equations developed by Parsons et al. (2013) in New York State of alfalfa-grass mixtures samples grown in Québec. a) equation 30, [RFQ = 420 - (91.8 × GFRAC) - (0.209 × GDD0) - (1.19 × AMAXHT)]; b) equation 26 [RFQ = 353 - (72.1 × GFRAC) - (2.21 × AMAXHT)]. AMAXHT, alfalfa maximum height in centimeters; GFRAC, proportion of grass within samples based on the DM weight (e.g., 0.1 or 0.6); GDD0, growing degree days based 0°C; AMAXSTAGE, Alfalfa maximum stage based on Kalu and Fick (1981). grass maximum height in centimeters. The solid line indicates the ideal 1:1 relationship while the dotted line represents the regression line.

4.2. Objective 2. Development of predictive equations of forage nutritive attributes for multiple growth cycles of mixed alfalfa-grass stands.

We successfully created two-, three-, and four-variable predictive equations to estimate concentrations of aNDFom and ADFom along with NDFdom, IVTDom, RFV, RFQ, and forage yield during the spring growth and the first summer regrowth that met our *a priori* defined threshold of an $R^2 \geq 0.75$. Of all the equations created (data not shown), the single best two-, three-, and four-variable predictive equations were selected based on the R^2, PRESS, and RMSE, while balancing the ease of determining the variables within the equation (Table 4.2). In general, as equations became more complex, the resulting R^2 values increased and they became better at predictions (PRESS and RMSE decreased) for all of the nutritive attributes of interest.

The most prevalent variable to appear in our equations was AMAXHT (alfalfa maximum height, cm); a variable that was previously reported in literature to also be important in other equations used to predict the nutritive value of alfalfa (e.g., Hintz and Albrecht,1991; Cherney, 1995; Parsons et al., 2006a; Andrzejewska et al., 2014). Alfalfa maximum height was an important variable in the majority of our equations with the exception of those for CP concentration. This variable was also reported to be important in predictive equations of alfalfa-grass mixtures previously developed for the first growth in New York State (Parsons et al., 2006b, 2013). Another variable of importance, based on the number of appearances in our equations, relates to the botanical composition of the forage mixture, being either GFRAC (i.e., the grass contribution to the total biomass) or AFRAC (i.e., the alfalfa contribution to the total biomass); this has been discussed previously in the literature (Parsons et al. 2006b, 2013). A botanical composition variable appears in all or the majority of our equations with the exception of the ADFom and forage yield predictive equations. 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), also appeared in the majority of our equations, especially the three- and four-variable equations, with the exception of the aNDFom equation. Other independent variables appear in other equations, but their frequency was lower than the previously enumerated variables.

4.2.1. Neutral detergent fibre concentration corrected for the organic matter content of the residue (aNDFom)

The best two-, three-, and four-variable equations for predicting aNDFom of alfalfa-grass mixtures during the spring growth and the first summer regrowth were equations 1, 2, and 3 (Table 4.2) with R^2 ranging from 0.78 to 0.82. These R^2 values were considered successful by our *a priori* defined minimum coefficient of determination ($R^2 \geq 0.75$) and were comparable to some R^2 values reported in the literature (Parsons et al., 2013). The RMSE was reduced from 32.1 to 29.3 g kg^{-1} DM as more variables were added; this is again in agreement with previous reports (Parsons et al., 2006b, 2013). Our observed mean 95% confidence interval (CI) decreased when comparing the two- and four-variable equations (63.0 to 57.6 g kg^{-1} DM) while the PRESS values decreased as more variables were added to the predictive equations.

4.2.2. Acid detergent fibre concentration corrected for the organic matter content of the residue (ADFom)

For ADFom concentration, the R^2 increased from 0.77 to 0.81 as more variables were used in equations (Table 4.2); this has also been reported in the literature for other predictive equations (Hintz and Albrecht, 1991; Parsons et al., 2013). In addition, as equations became more complex, the RMSE decreased from 21.2 to 19.7 g kg^{-1} DM and the mean 95% CI decreased from 41.7 to

38.7 g kg^{-1} DM. The RMSE values we observed are comparable to values previously reported in the literature (Hintz and Albrecht, 1991; Parsons et al., 2013).

4.2.3. *In vitro* neutral detergent fibre digestibility (NDFdom) and *in vitro* true digestibility (IVTDom) corrected for the organic matter content of the residue

The R^2 values we observed for our best predictive equations of NDFdom ranged between 0.80 and 0.84 (Table 4.2), and were better than those previously reported by Parsons et al. (2013) ($0.60 < R^2 < 0.77$) for predicting NDFdom for alfalfa-grass mixtures during first growth only. The RMSE decreased when the number of variables in equations increased from two to four. However, our RMSE values, even for the equation with four variables, were higher than those reported by Parsons et al. (2013) (28.7 vs. 29.0 g kg^{-1} DM) despite their equations having lower R^2 values. Again, as with the other variables, we observed a decrease in the mean 95% CI from 79.7 to 72.0 g kg^{-1} DM when comparing the two- and four-variable equations.

For IVTDom equations, very similar trends were observed with the R^2 increasing from 0.77 to 0.82 (Table 4.2), the RMSE decreasing from 25.2 to 22.2 g kg^{-1} DM, and the mean 95% CI decreased from 49.4 to 43.7 g kg^{-1} DM as the number of variables in the equation increased. To our knowledge, we are the first to attempt the development of predictive equations for forage IVTDom.

4.2.4. RFV and RFQ

The best two-, three-, and four-variable equations for RFV and RFQ had R^2 values that ranged between 0.76 and 0.80, which was comparable to values reported by Parsons et al. (2013) for some of their equations predicting these attributes of alfalfa-grass mixtures during spring growth.

The RMSE values we observed were similar to those they reported for RFQ, but were lower than those they reported for RFV (Parsons et al., 2013) despite the fact that our equations were developed for use during both the spring growth leading to the first harvest and the first summer regrowth leading to the second harvest.

4.2.5. Other attributes

None of the predictive equations we developed for CP met our minimum threshold of $R^2 \geq 0.75$, the highest value observed being 0.71 (Table 4.2). Such low values are comparable to what has been reported in the literature in previous attempts to develop and test predictive equations for the prediction of CP concentration in pure alfalfa samples (Fick and Janson, 1990; Hintz and Albrecht, 1991). Finally, equation development for forage yield was only partially successful. None of the two-variable equations met our minimum threshold for the coefficient of determination ($R^2 \geq 0.75$), however, the evaluated three- and four-variable equations did (i.e., with R^2 of 0.76 and 0.77, respectively). Unfortunately, the values for both the RMSE (895 and 873 kg DM ha^{-1}) and the mean 95% CI (1759 and 1717 kg DM ha^{-1}) suggest that the usability of these equations would be limited considering that the average annual forage yield in the Province of Québec ranges from 2,784 to 7,555 kg DM ha^{-1}, depending on the region, in a predominantly three cut system (FADQ, 2016).

4.2.6. Further discussion

The results we observed for our predictive equations were difficult to compare to the literature as, to our knowledge, this was the first attempt to develop predictive equations to predict the nutritive attributes of mixed alfalfa-grass samples taken either during the spring growth or the first summer regrowth. There is published literature on predictive equations for mixed alfalfa-grass fields but these are limited to the first growth (Parsons et al., 2006b and 2013); there were also predictive equations that were developed based on multiple growth cycles of pure alfalfa stands (Hintz and Albrecht, 1991) but none that combined the two concepts of mixed stands and multiple growth cycles. We would expect that the additional complexity resulting from the addition of the grass component would lead to equations with greater variability; this was confirmed by our results. Indeed, for example we observed lower R^2 and higher RMSE for our equations predicting aNDFom concentration of alfalfa-grass mixtures than those reported by Hintz and Albrecht (1991) for their equation predicting aNDF concentration in pure alfalfa fields.

As mentioned in earlier sections, when comparing our results to those of Parsons et al. (2006b, 2013), we generally found that our equations had lower R^2 and higher RMSE. Some of these differences could be attributed to the fact that our equations were developed with samples from both the spring growth and the first summer regrowth, unlike Parsons et al. (2006b, 2013) whom developed equations using only samples from the spring growth. We observed that if new predictive equations were created using only our data from the initial spring growth samples, our R^2 and RSME values were closer to those reported by Parsons et al. (2006b, 2013) (data not shown). This led us to believe that differences in regrowth patterns of different forage species upon the first harvest may have had an impact on predictions of their nutritive value. This is plausible as the two grass species we used differ in their development upon the spring harvest with tall fescue not producing reproductive structures during its regrowth (Wolf et al., 1979), while timothy has a regrowth comparable to the spring growth (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 cool season grasses (Van Soest, 1985). This may have an impact on how well the maximum height variables link to the nutritive attributes when using this variable during the first summer regrowth. While this is a general trend of forages, it is possible that weather variability from year to year will also impact the initial spring growth and the subsequent regrowth of forages and their nutritive value (Van Soest, 1985).

Table 4.2. Results of multiple regression equations created using data from three sites in the province of Québec (Sainte-Anne-de-Bellevue, Levis, and Normandin) and from the first and second growth cycles of mixed alfalfa-grass experimental plots to predict neutral detergent fibre (aNDFom; g kg^{-1} DM), acid detergent fibre (ADFom; g kg^{-1} DM), *in vitro* neutral detergent fibre digestibility based on 48hour incubation (NDFdom; g kg^{-1} aNDF), *in vitro* true digestibility based on 48 hour incubation (IVTD; g kg^{-1} DM), all corrected for the organic matter content of the residues, crude protein (CP; g kg^{-1} DM), relative feed value (RFV), relative feed quality (RFQ), and forage yield (kg DM ha^{-1}) (n= 1156)

Equations	**R^2**	**RMSE (g kg^{-1})**	**NRMSE (%)**	**PRESS**	**Mean 95% CI**
EQ1: aNDFom= 384.15 + (2.60 × AMAXHT) - (230.62 × AFRAC)	0.78	32.0	10.1	1192126	63.0
EQ2: aNDFom= 356.26 + (1.79 × AMAXHT) + (0.93 × GMAXHT) - (202.78 × AFRAC)	0.81	30.1	9.6	1049223	59.1
EQ3: aNDFom= 352.10 + (6.88 × AMAXSTAGE) + (1.34 × AMAXHT) + (1.05 × GMAXHT) - (205.23 × AFRAC)	0.82	29.3	9.3	995131	57.6
EQ4: ADFom= 159.18 + (1.29 × AMAXHT) + (0.83 × GMAXHT)	0.77	21.2	11.2	522056	41.7
EQ5: ADFom= 154.78 + (5.42 × AMAXSTAGE) + (0.93 × AMAXHT) + (0.93 × GMAXHT)	0.79	20.5	10.8	488582	40.3
EQ6: ADFom= 143.60 + (0.90 × AMAXHT) + (0.77 × GMAXHT) + (22.56 × GGRP) + (0.10 × GDD5)	0.81	19.7	10.4	450745	38.7
EQ7: NDFdom= 1068.54 - (0.37 × GDD0) - (276.05 × AFRAC)	0.80	40.6	9.2	1908526	79.7
EQ8: NDFdom= 829.46 - (1.64 × AMAXHT) - (0.22 × GDD0) + (250.15 × GFRAC)	0.83	37.1	7.9	1601547	73.0
EQ9: NDFdom= 934.28 - (1.55 × AMAXHT) - (0.23 × GDD0) + (142.30 × GFRAC) - (112.24 × AFRAC)	0.84	36.6	7.9	1556058	72.0
EQ10: IVTDom= 1017.86 - (7.15 × AMAXSTAGE) - (1.96 × AMAXHT)	0.77	25.2	10.6	733994	49.4
EQ11: IVTDom= 1011.53 - (1.36 × AMAXHT) - (0.13 × GDD0) + (42.28 × GFRAC)	0.80	23.2	9.6	625199	45.6
EQ12: IVTDom= 1016.36 - (0.90 × AMAXHT) - (0.73 × GMAXHT) + (40.53 × GGRP) - (0.16 × GDD5)	0.82	22.2	9.9	573637	43.7
EQ13: CP= 339.60 - (0.19 × GDD0) - (77.00 × GFRAC)	0.70	22.6	12.4	594078	44.5
EQ14: CP= 265.14 - (2.66 × AMAXSTAGE) - (0.17 × GDD0) + (80.30 × AFRAC)	0.71	22.5	12.2	587031	44.2
EQ15: CP= 302.26 - (2.73 × AMAXSTAGE) - (0.17 × GDD0) - (40.77 × GFRAC) + (42.38 × AFRAC)	0.71	22.4	12.0	581805	44.0
EQ16: RFV= 164.50 - (1.17 × AMAXHT) + (89.87 × AFRAC)	0.76	14.2	10.9	232539	27.8
EQ17: RFV= 184.97 - (0.67 × GMAXHT) - (0.10 × GDD5) + (66.45 × AFRAC)	0.79	13.4	9.8	207701	26.3
EQ18: RFV= 177.93 - (3.62 × AMAXSTAGE) - (0.61 × AMAXHT) - (0.44 × GMAXHT) + (77.95 × AFRAC)	0.80	12.9	9.7	194315	25.4
EQ19: RFQ= 280.18 - (1.00 × GMAXHT) - (0.17 × GDD5)	0.77	19.4	11.8	436743	38.1
EQ20: RFQ= 252.91 - (1.13 × AMAXHT) - (0.09 × GDD0) + (41.30 × AFRAC)	0.78	19.0	10.9	417005	37.3
EQ21: RFQ= 293.77 - (0.63 × AMAXHT) - (0.64 × GMAXHT) - (33.08 × GGRP) - (0.13 × GDD5)	0.80	18.2	10.9	385278	35.8
EQ22: Yield= 3623.68 + (79.82 × AMAXHT) - (29.29 × JULIAN)	0.74	928.6	10.9	999450399	1824.2
EQ23: Yield= 2555.73 + (62.01 × AMAXHT) + (20.45 × GMAXHT) - (24.64 × JULIAN)	0.76	895.0	11.2	929729828	1759.0
EQ24: Yield= 2264.61 + (50.18 × AMAXHT) + (15.43 × GMAXHT) - (24.17 × JULIAN) + (2.21 × GDD0)	0.77	873.4	11.3	885888092	1717.3

R^2: coefficient of determination; RMSE: root mean square error; NRMSE: normalized root mean square error; PRESS: Predictive sum of squares; and Mean 95% confidence interval (CI), is the range that the predicted result will fall within 95% of the time.

AMAXHT, alfalfa maximum height (cm); GMAXHT, grass maximum height (cm); AMAXSTAGE, alfalfa maximum staged based on Kalu and Fick (1981); GDD0, growing degree days base 0°C; GDD5, growing degree days base 5°C; AFRAC, alfalfa fraction of sample written as a decimal; GFRAC, grass fraction of sample written as a decimal; and GGRP, grass fraction group written as a decimal (i.e., 0.16, 0.20).

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4.3. Objective 3. Evaluating the use of predictive equations of forage nutritive attributes on commercial farms across Québec.

Some of the most promising predictive equations developed in section 4.2. (Table 4.2) were evaluated for their potential in predicting nutritive attributes of alfalfa-grass mixtures using data collected in fields of commercial farms from twelve administrative regions of Québec (i.e., Abitibi-Témiscamingue, Bas-Saint-Laurent, Capitale-Nationale, Centre-du-Québec, Chaudière-Appalaches, Estrie, Gaspésie-Îles-de-la-Madeleine, Lanaudière, Laurentides, Montérégie-Ouest, Mauricie, and Outaouais). The goal was to determine how well these equations perform in non-research situations, using samples and data collected from first growth alfalfa-grass mixtures taken by many participants, who ranged in experience working with forages and in determining the various variables required in those predictive equations. As for previous objectives of this project, we required the relationship between values generated using our predictive equations and values measured in the laboratory to have r^2 values of at least 0.75.

<u>4.3.1. Initial assessment using all participants' data</u>

Results for the predictive equations evaluated overall did not meet our minimum criteria; all of the equations resulting in r^2 lower than 0.75 for relationships between values they generated and observed values (Table 4.3). In summary, more complex equations with three or four variables overall performed better than the two-variable equations. The highest r^2 values observed for each nutritive attribute were as follows: aNDFom, 0.67; ADFom, 0.63; NDFdom, 0.57; IVTDom, 0.61; and RFV, 0.67, and were associated with four-variable equations, except in the case of RFV for which it was observed for a three-variable equation. The lowest RMSE values of each nutritive attribute are: aNDFom, 42.87 g kg^{-1} DM; ADFom, 28.29 g kg^{-1} DM; NDFdom, 57.23 g

kg^{-1} aNDF; IVTDom, 33.31 g kg^{-1} DM; and RFV, 16.75. The slope was significantly different from the ideal (slope = 1) for all equations except those equations for ADFom, which were not significantly different from the ideal. The intercept was significantly different from the ideal (intercept = 0) for all aNDFom and NDFdom equations and two of three equations predicting RFV, indicating a degree of bias; all equations predicting ADFom and IVTDom had intercepts that were not significantly different from the ideal while only one equation predicting RFV was not significantly different from the ideal indicating little to no bias for those equations evaluated.

A majority of the predictive equations evaluated included a botanical composition variable (AFRAC and/or GFRAC). It is a variable that has consistently been an important component of many equations developed to predict nutritive attributes of alfalfa-grass mixtures as previously reported by Parsons et al. (2006b, 2013) and in section 4.2. It is, however, very difficult to visually estimate this parameter as indicated by Parsons et al. (2006b, 2013) and supported by our own experiences. The predictive equations evaluated herein were developed with AFRAC and GFRAC being precisely determined; each botanical component being manually separated, dried, and weighted with exactitude using a scale (section 3.1.2). For the present objective, these two variables were, however, estimated visually to simplify the data collection by participants and as they did not all have access to driers and scales. It is the only of the variables used in the predictive equations evaluated that were collected or determined differently than in the equation development process.

4.3.2. Assessment using VNIRS determined forage proportions

In order to determine whether difference in the determination of AFRAC and GFRAC between objectives were one of the key reason for the significant departure in observed r^2 values between observed and predicted values, we used a previously developed visible near infrared reflectance spectroscopy (VNIRS)-based procedure to better determine AFRAC values from collected samples. This VNIRS-based procedure was previously developed using mixed alfalfa-grass samples from several studies conducted over a period of four years in several regions of Québec (data not shown). Once the AFRAC was determined using this approach, we calculated GFRAC by subtraction from 100 as participants collected samples mostly from field sections with minimal weed occurrence. This change also affected the variable GGRP as it was re-determined based on the new VNIRS-based GFRAC values. Not all sample values were impacted because the GGRP variable only had four possible values (20, 40, 60, or 80%). By inserting the VNIRS-determined AFRAC and GFRAC into the predictive equations in place of the values estimated visually by participants, while retaining all other field collected data, we were able to significantly improve the results of several equations that used these variables (Table 4.4). The modified GGRP had lesser impact on results as not all sample values were affected by the greater precision in GFRAC values.

Using field data collected by participants to the exception of AFRAC and GFRAC, which were instead determined by VNIRS, as well as the consequently impacted GGRP, significantly improved results for most equations evaluated. Using this approach, the resulting r^2 value for the relationship between observed and predicted values for aNDFom concentration and RFV, met our minimum threshold of $r^2 \geq 0.75$. For aNDFom, equations 2 and 3 (Table 4.4) had r^2 values of 0.79 and 0.80, and RMSE values of 34.28 and 33.12 g kg^{-1} DM, respectively; however, their slope remained significantly different from the ideal (slope=1) while the intercept was not significantly different from the ideal (intercept=0), indicating the presence of a bias. For RFV, equation 15 had an r^2 of 0.75 and RMSE of 14.51 while the slope and intercept were not significantly different from the ideal, indicating no presence of bias. We also observed improvement in the results of equations predicting NDFdom, but their r^2 values did not reach our minimum threshold.

The improvement in results for these equations was expected due to the importance of the AFRAC and GFRAC variables within the equations based on the magnitude of the coefficient value they are associated with. In the case of equations 1, 2, and 3 (predicting aNDFom concentration) and equations 7 and 8 (predicting NDFdom) (Table 4.4), the coefficients for either AFRAC or GFRAC were all greater than 200, while equation 9 had both AFRAC and GFRAC associated with coefficients greater than 100. This indicates that even a small change in the value of either AFRAC or GFRAC would lead to significant changes in the predicted values. For example, for equation 2 predicting aNDFom concentration, the coefficient is 202.78 for the AFRAC variable. In this case, 5 % change in the AFRAC value caused a 10.1 g kg^{-1} DM change in the estimated aNDFom value. This may seem relatively small, however, this would add to the error already inherently associated with the equation.

We only observed a slight improvement in results following the use of VNIRS estimated grass proportions for equation 6 which predicts ADFom concentration (Table 4.4). This equation, which relied on the GGRP variable, was only minimally impacted by the changes associated with an increase in the precision of the determination of the grass fraction. This is not surprising as changes to the 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 only changed the equation 6 predicted ADFom concentration by 4.5 g kg^{-1} DM.

In the case of equation 11 predicting IVTDom, despite the included variable GFRAC, no improvement in the r^2 value was observed following the use of VNIRS-determined GFRAC values (Table 4.4). This lack of improvement may be due to the fact that that the variable was associated with a relatively low coefficient in the predictive equation (i.e., 42.28). Impact on equation 12, which included the GGRP variable, was also minimal, probably for the same reasons as mentioned in the previous paragraph.

Although we saw improvement in the results for the majority of the predictive equations when using more precise VNIRS-determined GFRAC and AFRAC values, only three met our *a priori* defined minimum threshold of $r^2 \geq 0.75$ (Table 4.4). This led us to believe that some other factors must also be impacting our predictive equations results when used on commercial farms in a wide range of environments across Québec. These factors may be associated with data collection prior input into predictive equations including differences in users ability to determine certain variables (e.g., AMAXSTAGE), more errors during data recording by users, greater variation in field and soil management, grass species present, and distance to the nearest meteorological station impacting precision of GDD0 and GDD5 determinations. Other factors could also have affected laboratory measured values including sample handling, storage, and shipping. If samples were not put into a freezer as soon as possible upon collection or if they thawed during transportation this could have impacted laboratory measurements, and thus impacting correlations with predicted values.

Table 4.3. Validation of equations developed in Quebec to predict nutritive value attributes of mixed alfalfa-grass fields at the spring growth and first regrowth. Values inputted into equations for GFRAC and AFRAC were visually estimated. Data were collected from commercial farm fields located across 12 administrative regions of Québec. These equations predict the following nutritive attributes: neutral detergent fibre (aNDFom; g kg^{-1} DM), acid detergent fibre (ADFom; g kg^{-1} DM), *in vitro* neutral detergent fibre digestibility based on 48hour incubation (NDFdom; g kg^{-1} aNDF), *in vitro* true digestibility based on 48 hour incubation (IVTDom; g kg^{-1} DM), all corrected for the organic matter content of the residue, and relative feed value (RFV) (n= 315).

Equations (EQ)	Mean obs.	Mean pred.	r^2	RMSE	Slope coefficient (b)	SEb	Prob b = 1	Intercept coefficient (a)	SEa	Prob a = 0
EQ1: aNDFom= 384.15 + (2.60 × AMAXHT) - (230.62 × AFRAC)	457	407	0.56	49.57	0.84	0.041	0.001	116.32	17.22	< 0.0001
EQ2: aNDFom= 356.26 + (1.79 × AMAXHT) + (0.93 × GMAXHT) - (202.78 × AFRAC)	457	418	0.65	44.35	0.91	0.038	0.019	75.87	16.02	< 0.0001
EQ3: aNDFom= 352.10 + (6.88 × AMAXSTAGE) + (1.34 × AMAXHT) + (1.05 × GMAXHT) - (205.23 × AFRAC)	457	417	0.67	42.87	0.92	0.04	0.023	74.42	15.28	< 0.0001
EQ4: ADFom= 159.18 + (1.29 × AMAXHT) + (0.83 × GMAXHT)	309	316	0.61	29.02	0.99	0.04	0.87	-4.38	14.17	0.76
EQ5: ADFom= 154.78 + (5.42 × AMAXSTAGE) + (0.93 × AMAXHT) + (0.93 × GMAXHT)	309	315	0.63	28.31	1.00	0.04	0.92	-4.44	13.63	0.75
EQ6: ADFom= 143.60 + (0.90 × AMAXHT) + (0.77 × GMAXHT) + (22.56 × GGRP) + (0.10 × GDD5)	309	309	0.63	28.29	0.99	0.04	0.73	4.51	13.22	0.73
EQ7: NDFdom= 1068.54 - (0.37 × GDD0) - (276.05 × AFRAC)	656	658	0.47	63.71	0.75	0.05	< 0.0001	160.17	30.07	< 0.0001

EQ8: NDFdom= 829.46 - (1.64 × AMAXHT) - (0.22 × GDD0) + (250.15 × GFRAC)	656	665	0.57	57.23	0.84	0.04	< 0.0001	98.90	27.48	0.0004
EQ9: NDFdom= 934.28 - (1.55 × AMAXHT) - (0.23 × GDD0) + (142.30 × GFRAC) - (112.24 × AFRAC)	656	658	0.57	57.62	0.83	0.04	< 0.0001	110.16	27.25	< 0.0001
EQ10: IVTDom= 1017.86 - (7.15 × AMAXSTAGE) - (1.96 × AMAXHT)	805	857	0.56	35.54	0.96	0.05	< 0.0001	-13.01	40.94	0.75
EQ11: IVTDom= 1011.53 - (1.36 × AMAXHT) - (0.13 × GDD0) + (42.28 × GFRAC)	805	853	0.55	35.89	0.95	0.05	< 0.0001	-7.19	41.36	0.86
EQ12: IVTDom= 1016.36 - (0.90 × AMAXHT) - (0.73 × GMAXHT) + (40.53 × GGRP) - (0.16 × GDD5)	805	862	0.61	33.31	1.01	0.05	< 0.0001	-60.57	38.8	0.12
EQ13: RFV= 164.50 - (1.17 × AMAXHT) + (89.87 × AFRAC)	131	145	0.56	19.25	0.77	0.039	< 0.0001	19.15	5.7	0.0009
EQ14: RFV= 184.97 - (0.67 × GMAXHT) - (0.10 × GDD5) + (66.45 × AFRAC)	131	144	0.67	16.75	0.9	0.036	0.0039	2.02	5.23	0.70
EQ15: RFV= 177.93 - (3.62 × AMAXSTAGE) - (0.61 × AMAXHT) - (0.44 × GMAXHT) + (77.95 × AFRAC)	131	139	0.66	16.9	0.84	0.034	< 0.0001	13.3	4.85	0.0065

r^2: coefficient of determination; RMSE: root mean square error; AMAXHT, alfalfa maximum height (cm); GMAXHT, grass maximum height (cm); AMAXSTAGE, alfalfa maximum staged based on Kalu and Fick (1981); GDD0, growing degree days base 0°C; GDD5, growing degree days base 5°C; AFRAC, alfalfa fraction of sample written as a decimal; GFRAC, grass fraction of sample written as a decimal; and GGRP, grass fraction group, with group defined as the 20% interval closest to the GFRAC, written as a decimal (i.e., 0.16, 0.20).

Table 4.4. Validation of equations developed in Quebec to predict nutritive value attributes of mixed alfalfa-grass fields at the spring growth and first regrowth. Values inputted into equations for GFRAC, AFRAC, and GGRP were determined by VNIRS (visible near infrared reflectance spectroscopy). Only those equations that did change with the more precise data are shown. Data were collected from commercial farm fields located across 12 administrative regions of Québec. These equations predict the following nutritive attributes: neutral detergent fibre (aNDFom; g kg^{-1} DM), acid detergent fibre (ADFom; g kg^{-1} DM), *in vitro* neutral detergent fibre digestibility based on 48hour incubation (NDFdom; g kg^{-1} aNDF), *in vitro* true digestibility based on 48 hour incubation (IVTDom; g kg^{-1} DM), all corrected for the organic matter content of the residue, and relative feed value (RFV) (n= 315).

Equations (EQ)	Mean obs.	Mean pred.	r^2	RMSE	Slope coefficient (b)	SEb	Prob b = 1	Intercept coefficient (a)	SEa	Prob a = 0
EQ1: aNDFom= 384.15 + (2.60 × AMAXHT) - (230.62 × AFRAC)	457	424	0.74	38.27	1.06	0.04	0.077	5.67	15.31	0.71
EQ2: aNDFom= 356.26 + (1.79 × AMAXHT) + (0.93 × GMAXHT) - (202.78 × AFRAC)	457	434	0.79	34.28	1.08	0.03	0.015	-10.43	13.73	0.45
EQ3: aNDFom= 352.10 + (6.88 × AMAXSTAGE) + (1.34 × AMAXHT) + (1.05 × GMAXHT) - (205.23 × AFRAC)	457	433	0.80	33.12	1.07	0.03	0.026	-4.57	12.99	0.73
EQ6: ADFom= 143.60 + (0.90 × AMAXHT) + (0.77 × GMAXHT) + (22.56 × GGRP) + (0.10 × GDD5)	309	309	0.64	27.94	0.99	0.04	0.80	3.57	13.01	0.78
EQ7: NDFdom= 1068.54 - (0.37 × GDD0) - (276.05 × AFRAC)	656	680	0.58	56.57	0.99	0.05	0.76	-13.87	32.32	0.67
EQ8: NDFdom= 829.46 - (1.64 × AMAXHT) - (0.22 × GDD0) + (250.15 × GFRAC)	656	684	0.67	50.05	1.03	0.04	0.45	-48.96	27.99	0.08
EQ9: NDFdom= 934.28 - (1.55 × AMAXHT) - (0.23 × GDD0) + (142.30 × GFRAC) - (112.24 × AFRAC)	656	678	0.67	50.31	1.03	0.04	0.54	-39.36	27.82	0.16

EQ11: IVTDom= 1011.53 - (1.36 × AMAXHT) - (0.13 × GDD0) + (42.28 × GFRAC)	805	857	0.55	35.87	0.97	0.05	0.54	-25.57	42.26	0.55
EQ12: IVTDom= 1016.36 - (0.90 × AMAXHT) - (0.73 × GMAXHT) + (40.53 × GGRP) - (0.16 × GDD5)	805	861	0.61	33.51	1.02	0.05	0.67	-72.90	39.72	0.07
EQ13: RFV= 164.50 - (1.17 × AMAXHT) + (89.87 × AFRAC)	131	138	0.69	16.09	0.93	0.04	0.06	1.97	4.93	0.69
EQ14: RFV= 184.97 - (0.67 × GMAXHT) - (0.10 × GDD5) + (66.45 × AFRAC)	131	139	0.73	15.16	0.96	0.03	0.22	-2.16	4.68	0.65
EQ15: RFV= 177.93 - (3.62 × AMAXSTAGE) - (0.61 × AMAXHT) - (0.44 × GMAXHT) + (77.95 × AFRAC)	131	133	0.75	14.51	0.94	0.03	0.055	5.44	4.17	0.19

r^2: coefficient of determination; RMSE: root mean square error; AMAXHT, alfalfa maximum height (cm); GMAXHT, grass maximum height (cm); AMAXSTAGE, alfalfa maximum staged based on Kalu and Fick (1981); GDD0, growing degree days base 0°C; GDD5, growing degree days base 5°C; AFRAC, alfalfa fraction of sample written as a decimal; GFRAC, grass fraction of sample written as a decimal; and GGRP, grass fraction group, with group defined as the 20% interval closest to the GFRAC, written as a decimal (i.e., 0.16, 0.20).

5. Conclusions

5.1. Objective 1. Evaluating the potential of predictive equations of forage nutritive attributes developed in New York State for use in Québec.

Some of the equations developed by Parsons et al. (2006b, 2013) to predict forage quality attributes of alfalfa-grass mixtures appear to have potential for use in Québec and could become a useful tool to help them determine the optimal time to harvest their forage fields. Equations they developed in New York State to predict aNDFom, ADFom, RFV, and RFQ could be used in Quebec, but not equations developed to predict NDFdom. Caution is however required if using NYPEAQ equations in Québec as all had bias. This being said the statistics associated with the use of these equations in Québec are comparable to those reported in other validation studies of the use of equations used to predict forage quality attributes of pure alfalfa stands in regions outsides of which they were initially developed (e.g., Sulc et al., 1997; Hakl et al., 2010). The use of NYPEAQ equations are, however, limited to the spring growth, and predictive equations remains to be developed for certain nutritive attributes including crude protein, an important attribute used in ruminant diet formulation. The possibility of developing equations to predict forage quality attributes of alfalfa-grass mixtures for multiple regrowths should be explored. The results confirm our hypothesis that some NYPEAQ equations would meet the minimum threshold of $r^2 \geq 0.75$ and be considered valid in Québec.

5.2. Objective 2. Development of predictive equations of forage nutritive attributes for multiple growth cycles of mixed alfalfa-grass stands.

Creation of predictive equations capable of estimating some nutritive attributes of spring growth and first summer regrowth alfalfa-grass mixtures was successful (Table 4.2). Creating single equations able to predict the nutritive attributes of interest with data from locations with such varied growing conditions allowed for more robust equations, however, accuracy may have been sacrificed by not creating equations for specific regions. Accuracy was sacrificed in order to predict for both the spring growth and the first summer regrowth of mixed alfalfa-grass fields instead of creating equations to predict the spring growth or the first summer regrowth only. The samples selected to create the equations were mixtures of alfalfa and grass to ensure the generated equations were applicable to the needs of Québec producers, who predominately seed their fields with mixtures. The results observed were comparable to those reported by others in the scientific literature despite adding the extra complexity of predicting for alfalfa and grass mixtures and two growth periods instead of only one.

We were not successful in creating equations capable of predicting CP as the coefficient of determination of these equations did not meet our a priori minimum threshold ($R^2 \geq 0.71$) regardless of the number of variables included. We do not recommend using the forage yield predictive equations we developed. As we previously mentioned, the RMSE and mean 95% CI are too large to warrant use, especially when considering that the annual forage yield average in the Province of Québec ranges from 2,784 to 7,555 kg ha^{-1} in a predominantly three cut system (FADQ, 2016).

Our results confirmed our hypothesis that we could successfully create predictive equations for several nutritive attributes of importance. These equations had a lower correlation between predicted and measured values and a less accurate prediction than previous equations (i.e., original PEAQ and 2006b NYPEAQ equations), but were comparable to the 2013 NYPEAQ equations. This lower accuracy was due to the complexity of using sample data from alfalfa-grass mixtures gathered from multiple growth cycles compared to less complex equations.

5.3. Objective 3. Evaluating the use of predictive equations of forage nutritive attributes on commercial farms across Québec.

Using simple field measurements, predictive equations developed in Québec successfully predicted aNDFom concentration and RFV in the spring growth and the first summer regrowth of alfalfa-grass mixtures grown on commercial farms across Québec. The equations were successful only if GFRAC and/or AFRAC could be precisely determined (Table 4.4).

The successful predictive equations for aNDFom concentration were:

$$\text{aNDFom} = 356.26 + (1.79 \times \text{AMAXHT}) + (0.93 \times \text{GMAXHT}) - (202.78 \times \text{AFRAC}), \ (r^2 = 0.79)$$

$$\text{aNDFom} = 352.10 + (6.88 \times \text{AMAXSTAGE}) + (1.34 \times \text{AMAXHT}) + (1.05 \times \text{GMAXHT}) - (205.23 \times \text{AFRAC}), \ (r^2 = 0.80).$$

The RFV was successfully predicted using the equation;

$$\text{RFV} = 177.93 - (3.62 \times \text{AMAXSTAGE}) - (0.61 \times \text{AMAXHT}) - (0.44 \times \text{GMAXHT}) + (77.95 \times \text{AFRAC}), \ (r^2 = 0.75).$$

Equations predicting ADFom, and NDFdom, however, did not meet our minimum r^2 threshold and may be less adapted to use on commercial farms.

Our hypothesis was not originally confirmed as none of the predictive equations developed met our minimum r^2 threshold when using field data with visually estimated botanical composition of the samples. However, our hypothesis was confirmed when we used VNIRS determined botanical composition with field data, but only a few of the predictive equations met the minimum threshold once greater accuracy was gained for the botanical composition variables.

5.4. General conclusions and implication of results.

The present project demonstrated that the use in Québec of predictive equations for determining nutritive attributes of mixed alfalfa-grass mixtures is possible. These equations are based on the collection of relatively simple variables to be determined in the field by users and will help in determining optimal harvest time. We have shown that predictive equations developed in New York State could be used in the Province of Québec to predict aNDFom and ADFom concentrations for the spring growth of alfalfa-grass mixtures. We also developed new equations that could be used to determine these same nutritive attributes for both the spring growth and the first summer regrowth of alfalfa-grass mixtures. These new equations have good predictive statistics. The equation we developed to predict aNDFom [$aNDFom = 356.3 + (1.79 \times AMAXHT) + (0.93 \times GMAXHT) - (202.78 \times AFRAC)$] is particularly promising as its potential for use on commercial farms across Québec has been demonstrated. The choice of equations to select depends on a range of factors where a balance must be found between ease of use (few field variables easy to collect) and precision (more field variables sometimes harder to determine). The greatest limitation of our approach, and of evaluated and developed equations, consists in the fact that the quality of data predicted depends on the quality of the data collected in the field by users. The most promising equations include the need to determine either the proportion of grass or alfalfa in the mixture, field variables that are difficult to estimate visually for untrained users. It would thus be preferable to do a hand separation of samples in order to better determine with more precision this important variable.

Finally, it is important to mention that the use of these equations will only help users in determining the optimal time to harvest mixed alfalfa-grass fields. The use of predictive equations does not eliminate the need to determine the nutritive value of stored forage prior feeding for ration formulation. In addition, potential users must be aware of the limitation of the equations, namely that the results they provide will only be as good as the data collected and inputted.

6. Future Research

1) A year of testing to ensure that quick hand separation provides an accurate enough representation of the morphological compositions of the samples and therefore an accurate estimation of standing forage nutritive values.
2) Collection of a greater number of samples from commercial sites for both the spring growth and first summer regrowth. As well as collection of samples with alfalfa and grass species mixtures other than timothy and tall fescue to see if the model is capable of successful predicting their nutritive value.
3) Change the model from a static model based on 2015 and 2016 data to a rolling model that incorporates samples taken yearly. This helps increase accuracy of the model by incorporating any potential impact of meteorological effects that may occur from year to year.
4) Setup research fields in other Canadian provinces to test these newly created predictive equations to see if they can be used to successfully predict alfalfa and timothy or alfalfa and tall fescue mixtures standing nutritive values.

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8. Appendix

Objective 2 accompanying table

Table appendix 2.1. All two-, three-, and four-variable predictive equations generated from field data collected from mixed alfalfa-grass mixtures at three locations (Sainte-Anne-de-Bellevue, Levis, and Normandin) in the Province of Québec. Samples were collected during the spring growth and first summer regrowth.

Variables	Model equation	R^2	RMSE	NRMSE (%)	PRESS	Mean 95% CI
aNDFom	EQ1: aNDFom= 384.15 + (2.60 × AMAXHT) - (230.62 × AFRAC)	0.78	32.06	10.10	1192126	62.99
	EQ2: aNDFom= 167.86 + (2.53 × AMAXHT) + (227.26 × GFRAC)	0.75	33.84	10.88	1327560	66.48
	EQ3: aNDFom= 351.55 + (2.27 × GMAXHT) - (151.20 × AFRAC)	0.72	36.01	11.40	1503288	70.74
	EQ4: aNDFom= 356.26 + (1.79 × AMAXHT) + (0.93 × GMAXHT) - (202.78 × AFRAC)	0.81	30.07	9.61	1049223	59.10
	EQ5: aNDFom= 336.96 + (1.56 × GMAXHT) + (0.22 × GDD5) - (180.77 × AFRAC)	0.81	30.14	9.35	1054743	59.24
	EQ6: aNDFom= 364.01 + (1.88 × AMAXHT) + (0.10 × GDD0) - (215.71 × AFRAC)	0.80	30.85	9.43	1104549	60.63
	EQ7: aNDFom= 345.72 + (1.09 × AMAXHT) + (1.03 × GMAXHT) + (0.13 × GDD5) - (200.22 × AFRAC)	0.82	28.75	9.10	960610	56.54
	EQ8: aNDFom= 352.10 + (6.88 × AMAXSTAGE) + (1.34 × AMAXHT) + (1.05 × GMAXHT) - (205.23 × AFRAC)	0.82	29.27	9.30	995131	57.55
	EQ9: aNDFom= 345.3 + (1.37 × AMAXHT) + (0.80 ×GMAXHT) + (0.07 × GDD0) - (195.68 × AFRAC)	0.81	29.41	9.36	1005013	57.83
ADFom	EQ10: ADFom= 154.22 + (1.22 × GMAXHT) + (0.17 × GDD5)	0.78	21.14	11.41	518919	41.53
	EQ11: ADFom= 159.18 + (1.29 × AMAXHT) + (0.83 × GMAXHT)	0.77	21.22	11.19	522056	41.68
	EQ12: ADFom= 158.58 + (1.25 × AMAXHT) + (0.10 × GDD0)	0.76	21.89	11.45	555661	43.00

	EQ13: ADFom= 152.04 + (0.75 × AMAXHT) + (0.89 × GMAXHT) + (0.10 × GDD5)	0.80	20.05	10.62	467082	39.40
	EQ14: ADFom= 152.58 + (0.93 × AMAXHT) + (0.66 × GMAXHT) + (0.07 × GDD0)	0.79	20.30	10.74	478214	39.89
	EQ15: ADFom= 154.78 + (5.42 × AMAXSTG) + (0.93 × AMAXHT) + (0.93 × GMAXHT)	0.79	20.51	10.84	488582	40.31
	EQ16: ADFom= 169.18 + (0.97 × AMAXHT) + (0.71 × GMAXHT) + (0.10 × GDD5) - (29.01 × AFRAC)	0.81	19.43	10.29	439156	38.21
	EQ17: ADFom= 109.51 + (0.72 × AMAXHT) + (0.82 × GMAXHT) + (0.25 × JULIAN) + (0.07 × GDD0)	0.81	19.63	10.69	447964	38.59
	EQ18: ADFom= 143.31 + (0.91 × AMAXHT) + (0.76 × GMAXHT) + (0.10 × GDD5) + (24.08 × GFRAC)	0.81	19.63	10.44	448265	38.60
	EQ19: ADFom= 143.60 + (0.90 × AMAXHT) + (0.77 × GMAXHT) + (22.56 × GGRP) + (0.10 × GDD5)	0.81	19.69	10.44	450745	38.69
CP	EQ20: CP= 339.60 - (0.19 × GDD0) - (77.00 × GFRAC)	0.70	22.63	12.35	594078	44.46
	EQ21: CP= 267.21 - (0.19 × GDD0) + (75.30 × AFRAC)	0.70	22.63	12.38	594059	44.46
	EQ22: CP= 338.30 - (71.03 × GGRP) - (0.19 × GDD0)	0.68	23.39	13.85	634590	45.96
	EQ23: CP= 265.14 - (2.66 × AMAXSTAGE) - (0.17 × GDD0) + (80.30 × AFRAC)	0.71	22.49	12.21	587031	44.20
	EQ24: CP= 342.14 - (2.52 × AMAXSTAGE) - (0.17 × GDD0) - (81.72 × GFRAC)	0.71	22.51	12.20	587874	44.23
	EQ25: CP= 303.20 - (0.19 × GDD0) - (39.47 × GFRAC) + (38.48 × AFRAC)	0.71	22.53	12.22	589251	44.29
	EQ26: CP= 309.73 - (5.69 × AMAXSTAGE) + (0.19 × JULIAN) - (0.16 × GDD0) - (81.19 × GFRAC)	0.71	22.32	11.88	578276	43.88
	EQ27: CP= 234.63 - (5.70 × AMAXSTAGE) + (0.19 × JULIAN) - (0.16 × GDD0) + (79.67 × AFRAC)	0.71	22.32	11.92	578273	43.88
	EQ28: CP= 302.26 - (2.73 × AMAXSTAGE) - (0.17 × GDD0) - (40.77 × GFRAC) + (42.38 × AFRAC)	0.71	22.38	12.04	581805	44.01

RFV	EQ29: RFV= 164.50 - (1.17 × AMAXHT) + (89.87 × AFRAC)	0.76	14.16	10.85	232539	27.82
	EQ30: RFV= 246.68 - (1.15 × AMAXHT) - (86.08 × GFRAC)	0.74	14.85	11.65	255832	29.18
	EQ31: RFV= 245.34 - (1.14 × AMAXHT) - (81.84 × GGRP)	0.71	15.80	12.47	289553	31.05
	EQ32: RFV= 184.97 - (0.67 × GMAXHT) - (0.10 × GDD5) + (66.45 × AFRAC)	0.79	13.37	9.77	207701	26.28
	EQ33: RFV= 175.74 - (0.85 × AMAXHT) - (0.38 × GMAXHT) + (76.66 × AFRAC)	0.79	13.43	10.32	209370	26.40
	EQ34: RFV= 174.13 - (0.83 × AMAXHT) - (0.05 × GDD0) + (80.75 × AFRAC)	0.79	13.53	10.02	212432	26.59
	EQ35: RFV= 180.94 - (0.50 × AMAXHT) - (0.43 × GMAXHT) - (0.06 × GDD5) + (75.40 × AFRAC)	0.81	12.71	9.49	187742	24.99
	EQ36: RFV= 204.78 - (0.72 × AMAXHT) - (0.48 × GMAXHT) - (0.18 × JULIAN) + (78.88 × AFRAC)	0.80	12.93	9.89	194122	25.42
	EQ37: RFV= 177.93 - (3.62 × AMAXSTAGE) - (0.61 × AMAXHT) - (0.44 × GMAXHT) + (77.95 × AFRAC)	0.80	12.93	9.70	194315	25.43
RFQ	EQ38: RFQ= 280.18 - (1.00 × GMAXHT) - (0.17 × GDD5)	0.77	19.40	11.75	436743	38.12
	EQ39: RFQ= 277.67 - (0.71 × GMAXHT) - (0.14 × GDD0)	0.75	20.35	12.51	479775	39.97
	EQ40: RFQ= 276.41 - (0.88 × AMAXHT) - (0.12 × GDD0)	0.75	20.35	12.23	480402	39.99
	EQ41: RFQ= 264.40 - (0.93 × GMAXHT) - (0.19 × GDD5) + (25.77 × AFRAC)	0.78	18.83	10.91	411589	37.00
	EQ42: RFQ= 288.54 - (0.94 × GMAXHT) - (0.18 × GDD5) - (24.37 × GFRAC)	0.78	18.91	11.24	415198	37.16
	EQ43: RFQ= 252.91 - (1.13 × AMAXHT) - (0.09 × GDD0) + (41.30 × AFRAC)	0.78	18.96	10.87	417005	37.25
	EQ44: RFQ= 258.70 - (0.71 × AMAXHT) - (0.59 × GMAXHT) - (0.13 × GDD5) + (38.42 × AFRAC)	0.80	17.89	10.61	371673	35.17
	EQ45: RFQ= 294.16 - (0.66 × AMAXHT) - (0.62 × GMAXHT) - (0.13 × GDD5) - (35.20 × GFRAC)	0.80	18.09	10.92	380156	35.56

	EQ46: RFQ= 293.77 - (0.63 × AMAXHT) - (0.64 × GMAXHT) - (33.08 × GGRP) - (0.13 × GDD5)	0.80	18.21	10.94	385278	35.80
NDFdom	EQ47: NDFdom= 1068.54 - (0.37 × GDD0) - (276.05 × AFRAC)	0.80	40.57	9.18	1908526	79.70
	EQ48: NDFdom= 804.45 - (0.38 × GDD0) + (281.48 × GFRAC)	0.80	40.76	9.02	1926801	80.08
	EQ49: NDFdom= 815.45 - (3.26 × AMAXHT) + (217.17 × GFRAC)	0.78	42.14	8.39	2059895	82.79
	EQ50: NDFdom= 829.46 - (1.64 × AMAXHT) - (0.22 × GDD0) + (250.15 × GFRAC)	0.83	37.14	7.92	1601547	72.99
	EQ51: NDFdom= 1064.08 - (1.53 × AMAXHT) - (0.23 × GDD0) - (245.35 × AFRAC)	0.83	37.45	8.23	1628517	73.61
	EQ52: NDFdom= 1089.33 - (0.99 × GMAXHT) - (0.28 × GDD0) - (287.89 × AFRAC)	0.82	38.48	8.42	1720546	75.64
	EQ53: NDFdom= 934.28 - (1.55 × AMAXHT) - (0.23 × GDD0) + (142.30 × GFRAC) - (112.24 × AFRAC)	0.84	36.59	7.85	1556058	71.95
	EQ54: NDFdom= 829.52 - (1.38 × AMAXHT) - (0.43 × GMAXHT) - (0.21 × GDD0) + (260.19 × GFRAC)	0.84	36.82	7.80	1576614	72.39
	EQ55: NDFdom= 832.38 - (4.37 × AMAXSTAGE) - (1.54 × AMAXHT) - (0.20 × GDD0) + (243.75 × GFRAC)	0.83	36.92	7.88	1583619	72.59
IVTDom	EQ56: IVTDom= 1022.99 - (1.63 × AMAXHT) - (0.15 × GDD5)	0.78	24.18	10.79	677874	47.50
	EQ57: IVTDom= 1027.59 - (1.60 × AMAXHT) - (0.10 × GDD0)	0.78	24.38	10.72	689380	47.90
	EQ58: IVTDom= 1017.86 - (7.15 × AMAXSTAGE) - (1.96 × AMAXHT)	0.77	25.16	10.58	733994	49.43
	EQ59: IVTDom= 1063.52 - (1.18 × GMAXHT) - (0.23 × GDD5) - (57.71 × AFRAC)	0.80	23.14	10.70	621947	45.48
	EQ60: IVTDom= 1008.10 - (1.17 × GMAXHT) - (0.24 × GDD5) + (58.50 × GFRAC)	0.80	23.16	10.50	623080	45.52

	EQ61: IVTDom= 1011.53 - (1.36 × AMAXHT) - (0.13 × GDD0) + (42.28 × GFRAC)	0.80	23.21	9.64	625199	45.62
	EQ62: IVTDom= 1015.48 - (0.86 × AMAXHT) - (0.76 × GMAXHT) - (0.17 × GDD5) + (44.27 × GFRAC)	0.82	22.00	9.79	562758	43.26
	EQ63: IVTDom= 1056.79 - (0.84 × AMAXHT) - (0.77 × GMAXHT) - (0.17 × GDD5) - (42.77 × AFRAC)	0.82	22.08	9.93	566582	43.41
	EQ64: IVTDom= 1016.36 - (0.90 × AMAXHT) - (0.73 × GMAXHT) + (40.53 × GGRP) - (0.16 × GDD5)	0.82	22.22	9.90	573637	43.68
Forage yield	EQ65: Yield = 3623.68 + (79.82 × AMAXHT) - (29.29 × JULIAN)	0.74	928.56	10.85	999450399	1824.21
	EQ66: Yield = -1695.92 + (42.53 × AMAXHT) + (37.11 × GMAXHT)	0.67	1033.29	14.44	1238811463	2029.97
	EQ67: Yield = -1123.33 + (60.07 × AMAXHT) + (75.70 × GMAXSTG)	0.66	1051.01	8.43	1292855563	2064.77
	EQ68: Yield = 2921.45 + (59.26 × AMAXHT) - (27.25 × JULIAN) + (2.81 × GDD0)	0.76	891.27	10.91	921514108	1751.72
	EQ69: Yield = 2555.73 + (62.01 × AMAXHT) + (20.45 × GMAXHT) - (24.64 × JULIAN)	0.76	894.96	11.19	929729828	1758.97
	EQ70: Yield = 3817.50 + (81.62 × AMAXHT) - (26.57 × JULIAN) - (1299.28 × AFRAC)	0.75	898.02	10.73	935777636	1764.99
	EQ71: Yield = 2264.61 + (50.18 × AMAXHT) + (15.43 × GMAXHT) - (24.17 × JULIAN) + (2.21 × GDD0)	0.77	873.38	11.25	885888092	1717.30
	EQ72: Yield = 3199.48 + (64.47 × AMAXHT) - (25.58 × JULIAN) + (2.28 × GDD0) - (981.23 × AFRAC)	0.77	874.76	10.77	888570500	1720.00
	EQ73: Yield = 2295.22 + (63.90 × AMAXHT) - (25.69 × JULIAN) + (2.32 × GDD0) + (962.00 × GFRAC)	0.77	845.80	10.48	890625751	1722.05

R^2: Pearson coefficient of determination; R^2_{Pred}: Pearson coefficient of determination of prediction; RMSE: root mean square error; NRMSE: normalized root mean square error; PRESS: Predictive sum of squares.

AMAXHT, alfalfa maximum height (cm); GMAXHT, grass maximum height (cm); AMAXSTAGE, alfalfa maximum staged based on Kalu and Fick (1981); GDD0, growing degree days base 0°C; GDD5, growing degree days base 0°C; AFRAC, alfalfa fraction of sample written as a decimal; GFRAC, grass fraction of sample written as a decimal; and GGRP, grass fraction group written as a decimal (i.e., 0.16, 0.20).