High Quality Forages: How Sweet Should They Be?

Robert Berthiaume¹, Gaëtan F. Tremblay², Gilles Bélanger², Annick Bertrand², Yves Castonguay², Réal Michaud², Sophie Pelletier², André Brito³, Carole Lafrenière⁴, and Guy Allard⁵

Email: rberthiaume@valacta.com

■ Take Home Messages

- The diurnal increase of non-structural carbohydrate (NSC) concentration in alfalfa and timothy varies with growth cycles and sites (1.6 to 4.2% dry matter (DM)).
- This increase is accompanied by decreases in N, ADF, and NDF concentrations and by increases in *in vitro* DM digestibility.
- Greatest NSC concentrations are reached between 11 to 13 h after sunrise.
- Red clover and tall fescue forages have the greatest (9.4% DM) NSC concentration.
- Concentration of NSC of all species tested increases with PM cutting.
- The concentration of NSC in alfalfa can be improved (+9%) after one cycle of genetic selection.
- Concentration of NSC remains greater in PM- compared to AM-cut alfalfa throughout the wilting period when forage is mown into wide swaths.
- Increasing forage NSC concentrations produces a more glucogenic rumen fermentation (in vitro).
- Forages with more NSC are more digestible and enhance the synthesis of microbial protein in the rumen (*in vitro*).

¹Valacta, 555 boul. des Anciens-Combattants, Sainte-Anne-de-Bellevue QC H9X 3R4

²Agriculture and Agri-Food Canada, Soils and Crops Research and Development Centre, Québec, QC, G1V 2J3 ³University of New Hampshire, Department of Biological Sciences, Durham, NH, USA 03824

⁴Dairy and Swine Research and Development Centre, Sherbrooke, QC, J1M 1Z3

⁵Plant Science Department, Université Laval, Québec, QC, G1V 0A6

Dairy cows ingest more DM when fed forages with more NSC leading to a more efficient use of dietary N and to an increase in milk production (5-10%).

With the large increases in the cost of fertilizers and concentrates, small changes in plant composition can have large impacts on the sustainability of milk production.

Introduction

There are a variety of carbohydrates in feeds that will matter nutritionally to the animal. Sugars, starch, fructans, pectins, and neutral detergent fiber (NDF) make up the majority of carbohydrates in forages. Carbohydrates such as starch and sugars can be digested directly by the animal, whereas fructans, pectins, and NDF can only be digested by gut microbes. Analyzing forages to describe the content of carbohydrates that are nutritionally relevant to animals has been challenging and has led to a lot of confusion. Water-soluble carbohydrates (WSC) include sugars, oligosaccharides, and fructans whereas total nonstructural carbohydrates (NSC) include WSC + starch. Nonstructural carbohydrates should not be confounded with nonfiber carbohydrates (NFC) which are calculated to include all carbohydrates not in NDF.

It is well documented that the concentration of sugars in forages, referred to as nonstructural carbohydrates (NSC) in the text, varies diurnally because of the plants' potential to accumulate carbohydrates during the day (Gordon, 1996; Burns et al., 2007). Alfalfa is the most widely cultivated forage legume in Canada because of its high yield and crude protein (CP) concentration. However, between 44 and 87% of the CP in alfalfa silage is degraded to non-protein nitrogen (NPN) in the silo (Papadopoulos and McKersie, 1983; Muck, 1987) leading to excessive ammonia formation in the rumen. Thus, an increase in alfalfa NSC to balance the supplies of fermentable energy and rumen degradable protein (RDP) in the rumen may enhance ammonia capture by ruminal microbes and improve nitrogen (N) utilization in dairy cows.

In Europe, reducing the ratio of CP to soluble sugars by feeding perennial ryegrass cultivars bred for high WSC significantly improved N utilization and milk yield in both early and late-lactation dairy cows (Miller et al., 2001; Moorby et al., 2006). Daytime cutting management (PM- vs. AM-cut) also offers an opportunity to increase NSC concentration of grasses (tall fescue) and legumes (alfalfa) as reported by Fisher et al. (1999, 2002). Investigating the variation in ruminant preference for alfalfa hays, Fisher et al. (2002) and Burns et al. (2005) observed that shifting hay mowing from sunup to sundown increased dry matter (DM) intake in trials conducted with sheep, goats, and steers. Notwithstanding these few examples, the potential for improving the

nutritional value and efficiency of N utilization by feeding alfalfa with improved NSC concentration to lactating dairy cows remains largely unexploited.

Therefore, we hypothesized that feeding high NSC forage would improve N utilization, DM intake, and milk yield in dairy cows. Our objective was to increase the energy content of forages with the overall goal of improving dairy cow performance via increased DM intake and N use efficiency. Specific objectives were 1) to develop crop management practices that favour the accumulation of energy in forages; 2) to evaluate the impact of a forage rich in energy on *in vitro* dry matter digestibility and microbial protein synthesis, and on intake, N utilisation, and performance of dairy cows.

■ Variations In Forage NSC Concentration from the Plant to the Mouth of The Animal

Diurnal Variations of NSC in Alfalfa (Morin et al., 2011) and Timothy (Morin et al., 2012b)

We studied the diurnal variations of NSC concentration and other nutritive value attributes in alfalfa to determine the best time during the day to cut alfalfa for maximizing NSC concentration. Field–grown alfalfa was cut every two hours between 0600 and 2000 h on six different days around the early flower stage of development during spring growth and summer regrowth at two sites in eastern Canada. Concentrations of NSC [soluble carbohydrates (SC) + starch], neutral detergent fiber (NDF), acid detergent fiber (ADF), and N, along with *in vitro* true digestibility (IVTD) of dry matter (DM) and *in vitro* digestibility of NDF (dNDF) were determined.

The alfalfa NSC concentration increased during the day in all growth cycles and sites due mostly to an increase in starch concentration (Figure 1). The diurnal increase of NSC concentration, however, varied with growth cycles and sites from 15.5 to 41.9g/kg DM, and it was accompanied by a decrease of 1 to 2 g/kg DM in N concentration. At the site where the increase in alfalfa NSC concentration was greater than 30 g/kg DM, concentrations of ADF and NDF also decreased by 9 to 27 g/kg DM while IVTD increased by 3 to 16 g/kg DM.

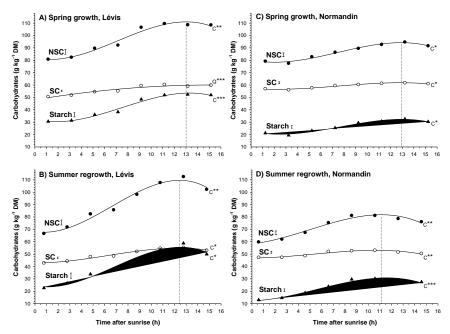


Figure 1. Diurnal variations in alfalfa concentrations of nonstructural carbohydrates (NSC = SC + starch), soluble carbohydrates (SC = sucrose + glucose + fructose + pinitol + maltose), and starch in spring growth (A) and summer regrowth (B) at Lévis, and spring growth (C) and summer regrowth (D) at Normandin (6–day averages with associated standard errors of the mean). Dotted lines represent time after sunrise when the NSC concentration reached its maximum value. Regression curve types were chosen based on the significance of the quadratic (Q) and cubic (C) components of time after sunrise with *, P < 0.05; **, P < 0.01; ***, P < 0.001 (Morin et al., 2011).

We also studied the diurnal variations of NSC concentration in timothy (Morin et al., 2012b). Greatest NSC concentrations were reached between 11 and

13 h after sunrise. Therefore, the best time during the day to cut alfalfa and timothy for maximizing NSC concentration in eastern Canada is between 1600 and 1800 h.

Time of Cutting and Species Affect Forage NSC (Pelletier et al., 2010)

The NSC concentration of several forage grass and legume species currently grown in Canada were compared. We also wanted to determine how variations of NSC concentration caused by time of cutting during the day differ among forage species and how these variations are related to other attributes

of forage nutritive value. Six grass and two legume species were cut at 0900 h (AM) and 1530 h (PM) in the spring growth and summer regrowth of two harvest years. The NSC concentration was estimated by the sum of sucrose, glucose, fructose, pinitol, fructans, and starch.

Red clover and tall fescue had the greatest NSC concentration [94.2 g/kg of DM across time of cutting and growth periods] whereas reed canarygrass had the lowest NSC concentration (65.5 g/kg DM) (Figure 2). Concentration of NSC of all species increased with PM-cutting but the extent of this increase varied among forage species. This increase, averaged across growth periods, went from 13% in smooth bromegrass (67.0–73.9 g/kg DM) to 68% in reed canarygrass (49.6–81.4 g/kg DM).

Increased NSC concentration with PM-cutting was associated with significant but small decreases in N, ADF, and NDF concentrations and a small increase in *in vitro* true DM digestibility (Pelletier et al., 2010a). We concluded that both species selection and PM-cutting can be used to increase forage NSC concentration.

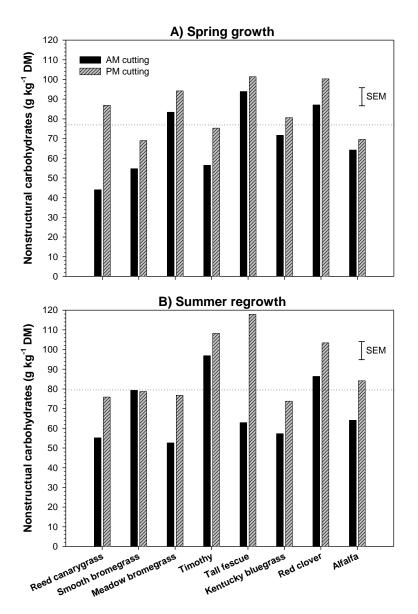


Figure 2. Concentrations of nonstructural carbohydrates [NSC, estimated by the sum of sucrose, glucose, fructose, pinitol (legumes), low and high degree of polymerization fructans (grasses), and starch] in spring growth and summer regrowth of six grasses and two legumes) cut in the morning (0800 h to 1000 h) or the afternoon (1500 h to 1615 h). Forages were grown in Normandin and values are averages of two production years (Pelletier et al., 2010a).

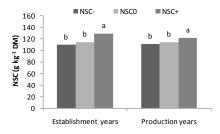
In a more recent trial comparing 18 binary forage mixtures of one legume (alfalfa, white clover, or birdsfoot trefoil) and one grass species (timothy, Kentucky bluegrass, tall fescue, orchardgrass, meadow bromegrass, or meadow fescue), Simili da Silva et al. (2012) reported that alfalfa-timothy (172 g/kg DM), alfalfa-Kentucky bluegrass (162 g/kg DM), birdsfoot trefoil-meadow fescue (161 g/kg DM), and alfalfa-meadow fescue (157 g/kg DM) mixtures had greater NSC concentrations than white clover-orchardgrass and white clover-timothy mixtures (125 g/kg DM). Mixtures of meadow fescue with any legume species, especially with alfalfa, provided the best combination of a greater ratio of WSC/CP (0.70), DM yield, and DM digestibility.

How Genetic Selection Affects NSC in Alfalfa (Chouinard-Michaud et al., 2010)

Forage NSC concentration can be improved via genetic selection but the extent of these effects is not well known under Canadian climatic conditions. Our objective was to determine, under field conditions, the effect of populations of alfalfa selected for high and low concentrations of NSC, cut in the morning or the afternoon, on NSC concentrations and other attributes of nutritive value.

Five hundred alfalfa (cv. AC Caribou) genotypes were harvested at three different times. We selected 10 genotypes with low NSC concentrations and 10 other genotypes with high NSC concentrations at the three harvests. A third group of 10 genotypes was randomly chosen. By intercrossing the 10 genotypes of each group, three alfalfa populations were obtained; NSC- (low NSC concentrations), NSC0 (control), and NSC+ (high NSC concentrations). The three populations were established in plots near Quebec City in 2006 and 2008, and cut in the AM (0900 h) and PM (1500 h) at the early flowering stage of development during two establishment years (one cut per year) and three production years (three cuts per year).

In the establishment years, NSC concentrations increased by 13% with NSC+ compared to NSC0 population and by 46% with PM- compared to AM-cuttings (Figure 3). In the production years, NSC concentrations increased by 6% with NSC+ compared to NSC0 population and by 37% with PM- compared to AM-cuttings (Figure 4). There was no interaction between populations and time of cutting. This increase with either PM-cutting or the NSC+ population was mostly due to an increase in starch concentration. In general, PM-cutting decreased ADF, NDF, and CP concentrations, and increased IVTD and dNDF. The NSC+ and NSC- populations did not differ for CP, ADF, NDF, IVTD, and dNDF.



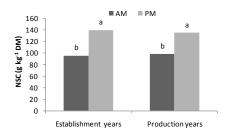


Figure 3. Concentrations of nonstructural carbohydrates (NSC = sucrose + glucose + fructose + pinitol + starch) in three alfalfa populations [NSC-, selected for low NSC concentration; NSC0, randomly selected (control); and NSC+, selected for high NSC concentration] cut in AM (0900 h) and PM (1500 h) during two establishment years (1 cut/year, n = 96) and three production years (3 cuts/year, n = 432) (Chouinard-Michaud et al., 2010).

Again, we concluded that alfalfa NSC concentrations can be increased by cutting the forage in the afternoon and via genetic selection, although this increase was more important with time of cutting (+ 42%) than with selection (+9%). Additional cycles of selection may further increase NSC concentrations and we are currently testing this hypothesis. Interestingly, the increase in NSC concentrations with PM-cutting was associated with a decrease in ADF and NDF concentrations, and an increase in IVTD and dNDF.

Variation of Alfalfa NSC Concentration during Wilting (Morin et al., 2012a)

As shown earlier, delayed cutting during the day increases NSC concentrations in forages. However, little is known about what happens to NSC concentration during wilting. Changes in NSC concentrations during wilting of PM- and AM-cut alfalfa were measured (Morin et al., 2012a; Tremblay et al. 2010). Field-grown alfalfa (cv. AC Caribou) was cut and left to wilt in narrow or wide swaths at the early flowering stage of development either at the end of the afternoon (PM) of a sunny day or the following morning (AM). Forage samples were taken at cutting times, then every two hours from 0830 h until the end of the second day, and every four hours thereafter. This 3-day experiment was conducted twice in the spring (12-14 June; 30 June-2 July) and summer (20-22 August; 26-28 August), and once in the fall (6-8 October) of 2008.

At cutting, alfalfa NSC concentration was, on average, 24% greater in PM-than in AM-cut alfalfa [+21% in spring (Fig. 4A), +27% in summer (Fig. 4B), and +23% in fall (Fig. 4C)]. Concentration of NSC remained greater in PM-(lines with circle symbols) compared to AM-cut alfalfa (lines with square symbols) throughout the wilting period (Figure 4). The rate of decrease in

alfalfa NSC concentration during wilting was lower in the summer and fall when drying conditions were superior. Wilting was effectively faster in the summer and fall. At the end of the second day, forage DM concentrations reached 26-34% in the spring, 34-41% in the summer, and 36-43% in the fall (see tables under graphs in Figure 4).

When wilting was fast (summer and fall) alfalfa NSC concentrations were, on average, 8% greater with wide swathing (solid lines) than with narrow swathing after cutting (dashed lines in Figure 4). We concluded that wide swathing helps preserve NSC concentration in alfalfa forage, especially when conditions allow fast wilting. Therefore, cutting alfalfa at the end of the afternoon (1600-1800 h) and leaving it to wilt in wide swaths maximizes NSC concentration in wilted forage when climatic and crop conditions favour rapid wilting.

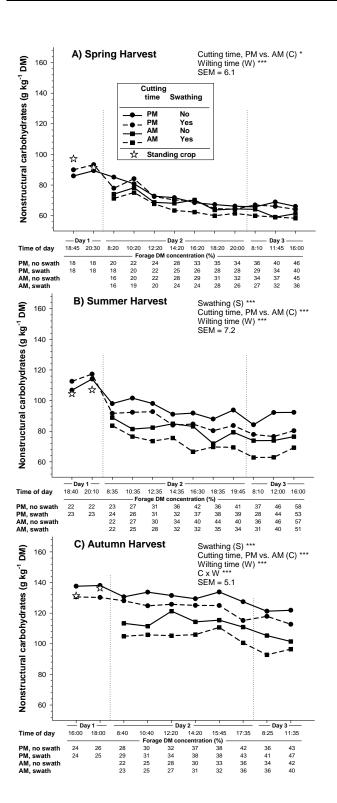


Figure 4. Evolution of nonstructural carbohydrate concentration during the wilting period of alfalfa cut at the end of a sunny day (PM) or the following morning (AM). This 3-day experiment was conducted twice in the spring (A) and summer (B), and once in the fall (C). Averages for each growth period are presented (Morin et al., 2012a).

Variation of NSC Concentration during Silage Fermentation

The evolution of the nonstructural carbohydrates during the first 128 days of silage fermentation was assessed with laboratory mini-silos using PM- and AM-cut alfalfa in spring growth and summer regrowth. The NSC concentration in alfalfa rapidly decreases during silage fermentation. Our preliminary analyses showed that when the difference in alfalfa NSC concentrations between PM- and AM-cutting at ensiling was relatively small (6 g/kg DM) in the spring growth, this difference completely disappeared after 128 days of silage fermentation. In the summer regrowth, the difference in alfalfa NSC concentration between PM- and AM-cutting at ensiling was greater (11 g/kg DM) and this difference did not completely disappear but was attenuated to 4 g/kg DM after 128 days of silage fermentation. The average DM concentration was 370 and 410 g/kg for the spring growth and summer regrowth silages, respectively.

Variations in Forage NSC Concentration from the Rumen to the Bulk Tank

Forage NSC Concentration Affects Rumen Microbial Protein Synthesis

Efficient microbial protein synthesis in the rumen requires a balanced supply of non-protein nitrogen (amino acids, peptides, and ammonia) and energy. Unfortunately, this is not the case with forages such as alfalfa. Peptides and ammonia from soluble leaf proteins are rapidly released by the action of plant and microbial proteases (Kingston-Smith and Theodorou, 2000) and the supply of rapidly available energy in the form of NSC tends to be limited. Hence, the micro-organisms have to rely on structural carbohydrates (cellulose and hemicellulose) which are broken down at a much slower rate. Consequently, this results in the loss of ammonia from the rumen and a reduction in efficiency of microbial protein synthesis. This problem is compounded in silages where a large proportion of NSC is used by the silage microflora to produce lactic acid and volatile fatty acids (VFA). Therefore, any treatment that increases the WSC concentration in forages without an increase in RDP should have beneficial effects. Table 1 summarizes the results of three different in vitro studies (Berthiaume et al, 2010 and unpublished) conducted to determine if genetic selection and PM-cutting to increase NSC would affect the rumen metabolism of forages.

	NSC (9	NSC (%)		_DMD ^z (%)		A:P		NH ₃ - N(mg/dL)	
	High ^y	Low	High	Low	High	Low	High	Low	
Alfalfa	13.3	7.1	nd	nd	2.7	3.9	58.4	63.6	
Alfalfa	17.9	7.4	74	65	3.0	3.6	26.0	33.6	
Timothy	13.7	9.6	79	81	2.9	3.1	15.3	49.4	

Table 1. Summary of three in vitro trials with high- and low-NSC forages

In general, feeding High-NSC forages increased DM digestibility and reduced both the acetate to propionate ratio and the concentration of ammonia in the rumen. Those results are evidence that the energy and protein contained in High-NSC forages were used more efficiently than low-NSC forages by the rumen bacteria.

What Do The Cows Think?

Our *in vitro* studies needed to be confirmed in vivo with dairy cows fed at greater feeding, which results in faster passage rates from the rumen. Hence, we used 16 late lactation dairy cows fed an all forage diet to investigate the effects of alfalfa daytime cutting management on ruminal metabolism, nutrient digestibility, N balance, and milk yield. Compared to AM baleage, PM baleage concentrations of starch, WSC, and NSC were increased by 50, 19, and 22%, respectively (Figure 5).

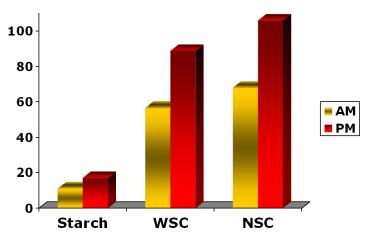


Figure 5. Effect of cutting time on alfalfa baleage NSC concentration (g/kg DM).

^zDMD = In vitro rumen Dry matter digestibility; A:P = Acetate to Propionate ratio.

^yHigh and Low refer to High- and Low-NSC alfalfa obtained using either genetic variability or time of cutting during the day. nd = not determined.

Rumen pH was significantly higher comparing PM- vs. AM-cut alfalfa at 2, 3, 4, 6, and 8 h post-feeding (Figure 6) whereas concentrations of rumen ammonia did not differ between forage treatments. This is contrary to *in vitro* data but it must be emphasized that in the controlled conditions of *in vitro* systems, there is no absorption of ammonia and/or recycling of urea, which are two important factors influencing the concentration of ammonia in the rumen.

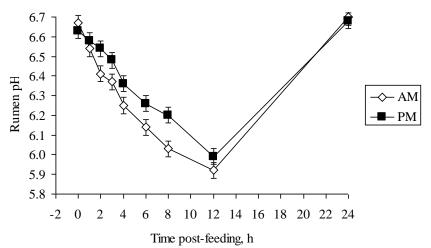
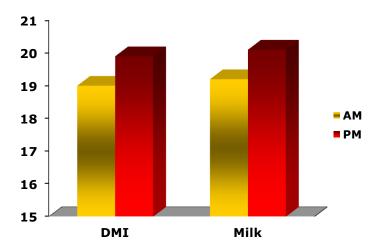


Figure 6. Diurnal variation of rumen pH in dairy cows fed baleage harvested from alfalfa cut at sundown (PM) or sunup (AM).

Dry matter intake and milk yield were both greater when feeding PM- rather than AM-cut alfalfa (Figure 7). Yields of milk components were also significantly higher when cows were fed PM- vs. AM-cut alfalfa baleage. Digestible organic matter (OM) intake was 0.8 kg/d higher when feeding PM-rather than AM-cut alfalfa, which corresponds to an increment of 13 MJ of metabolisable energy (ME) intake with the former treatment assuming an energy content of 16 MJ/kg of digestible OM. Considering that cows fed PM-cut alfalfa yielded 1.6 kg/d more energy corrected milk (ECM) than those fed AM-cut alfalfa, the marginal response in ECM to incremental metabolizable energy (ME) intake was 0.12 kg of ECM/MJ of ME.



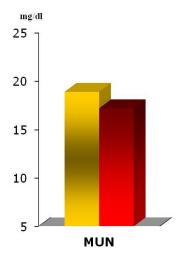


Figure 7. Effect of cutting time of alfalfa on DMI, milk production, and milk urea nitrogen (MUN).

Milk urea N was lower with feeding PM- vs. AM-cut alfalfa baleage suggesting improvement in N utilization by shifting alfalfa cutting from AM to PM. Increased forage NSC improved the capacity of ruminal microbes to capture ammonia and use it as a N source for growth and yield. Ammonia is often the main N source for microbial protein synthesis and it is essential for the growth of several species of ruminal bacteria. In fact, ruminal bacteria incorporated more ammonia N when cows were fed PM- vs. AM-cut alfalfa further explaining the enhanced ruminal outflow of bacterial non-ammonia nitrogen

(NAN) with PM-cut alfalfa. Finally, efficiency of N use was greater when cows were fed PM- rather than AM-cut alfalfa baleage. Further studies conducted with early and mid-lactation cows fed alfalfa (Brito et al., 2009), timothy (Brito et al., 2010) or perennial ryegrass (Miller et al., 2001 and Moorby et al., 2006) generally concurred and reported that lactating dairy cows eat more and produce more milk with High NSC forages (Table 2).

Table 2. Summary of four in vivo trials with high- and low-NSC forage

	NSC (%)		DMI ^z	DMI ^z (kg/d)		Milk (kg/d)		MUN (mg/dL)	
	High	Low	High	Low	High	Low	High	Low	
Brito et al.,									
2009	4.2	3.0	21.5	22.0	27.5	28.6	10.7	11.6	
Brito et al.,									
2010	7.7	6.0	19.0	18.2	22.6	21.8	19.6	20.0	
Miller et al.,									
2001	16.5	12.6	15.1	14.2	15.3	12.6	16.0	22.0	
Moorby et al.,									
2006	24.3	16.1	18.8	16.6	32.7	30.4	21.0	24.0	

^zDMI = Dry matter intake; MUN = milk urea N. Milk is not corrected for milk components.

As expected the response to sweet forages was less and more variable with high concentrate diets fed to cows in early lactation.

Conclusion

Feeding high NSC forage did improve N utilization, DM intake, and milk yield in mid and late lactation dairy cows. Both genetic selection and the development of crop management practices that favour the accumulation of energy in forages should be pursued. In the present climate of increasing demand and prices of grain and oilseed, producers must balance diets for optimum production while maximizing the use of home grown feeds and minimizing the use of inorganic fertilizers. Sweet forages should have a prominent place in a sustainable dairy production system.

Acknowledgements

The authors gratefully acknowledge the contribution of other research scientists: Doris Pellerin (Université Laval), Alain Fournier (MAPAQ), and Daniel Lefebvre (Valacta). The scientific contribution of other highly qualified personnel is also appreciated: Fadi Hassanat, postdoctoral fellow; Zhidong Nie and Chantale Morin, Ph.D. students; Caroline Chouinard-Michaud and Geneviève Régimbald, M.Sc. students.

The authors also acknowledge the financial support of Agriculture and Agri-Food Canada, FQRNT (Fond québécois de la recherche sur la nature et les technologies), Novalait inc., and MAPAQ (Ministère de l'agriculture, des pêcheries et de l'alimentation du Québec).

References

- Bélanger, G., C. Morin, G. F. Tremblay, A. Bertrand, Y. Castonguay, R. Michaud, R. Berthiaume et G. Allard. 2009. Diurnal variation of non-structural carbohydrate concentrations in alfalfa and timothy. Communication scientifique présentée au ASA-CSSA-SSSA International Annual Meetings, 1-5 nov., Pittsburgh, PA. http://a-c-s.confex.com/crops/2009am/webprogram/Paper54687.html
- Berthiaume, R., C. Benchaar, A. V. Chaves, G. F. Tremblay, Y. Castonguay, A. Bertrand, G. Bélanger, R. Michaud, C. Lafrenière, T. A. McAllister, and A. F. Brito. 2010. Effects of nonstructural carbohydrate concentration in alfalfa on fermentation and microbial protein synthesis in continuous culture. J. Dairy Sci. 93:693-700.
- Brito, A. F., G. A. Broderick, and S. M. Reynal. 2006. Effects of varying dietary ratios of alfalfa silage to corn silage on omasal flow and microbial protein synthesis in dairy cows. J. Dairy Sci. 89:3939-3953.
- Brito, A. F., G. A. Broderick, and S. M. Reynal. 2007. Effects of different protein supplements on omasal nutrient flow and microbial protein synthesis in lactating dairy cows. J. Dairy Sci. 90:1828-1841.
- Brito, A. F., G. F. Tremblay, A. Bertrand, Y. Castonguay, G. Bélanger, R. Michaud, H. Lapierre, C. Benchaar, H. V. Petit, D. R. Ouellet, and R. Berthiaume4. 2008. Alfalfa cut at sundown and harvested as baleage improves milk yield of late-lactating dairy cows. J. Dairy Sci. 91:3968-3982.
- Brito, A. F., G. F. Tremblay, H. Lapierre, A. Bertrand, Y. Castonguay, G. Bélanger, R. Michaud, C. Benchaar, D. R. Ouellet, and R. Berthiaume. 2009. Alfalfa cut at sundown and harvested as baleage increases bacterial protein synthesis in late-lactation dairy cows. J. Dairy Sci. 92:1092-1107.
- Burns, J. C., H. F. Mayland, and D. S. Fisher. 2005. Dry matter intake and digestion of alfalfa harvested at sunset and sunrise. J. Anim. Sci. 83:262-270.
- Burns, J. C., D. S. Fisher, and H. F. Mayland. 2007. Diurnal shifts in nutritive value of alfalfa harvested as hay and evaluated by animal intake and digestion. Crop Sci. 47:2190-2197.
- Chouinard-Michaud, C., R. Michaud, Y. Castonguay, A. Bertrand, G. Belanger, G. F. Tremblay, R. Berthiaume, and G. Allard. 2010. Time of cutting and genetic selection affect non-structural carbohydrates and some attributes of nutritive value in alfalfa. Scientific poster presented at the 2010 ASA-CSSA-SSSA International Annual Meetings, 31 Oct. 3 Nov., Long Beach, CA. Abstract 187-11.

- Fisher, D. S., H. F. Mayland, and J. C. Burns. 1999. Variation in ruminant's preference for tall fescue hays cut either at sundown or at sunup. J. Anim. Sci. 77:762-768.
- Fisher, D. S., H. F. Mayland, and J. C. Burns. 2002. Variation in ruminant preference for alfalfa hays cut at sunup and sundown. Crop Sci. 42:231-237.
- Moorby, J. M., R. T. Evans, N. D. Scollan, J. C. MacRae, and M. K. Theodorou. 2006. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Evaluation in dairy cows in early lactation. Grass Forage Sci. 61:52-59.
- Morin, C., G. Bélanger, G. F. Tremblay, A. Bertrand, Y. Castonguay, R. Drapeau, R. Michaud, R. Berthiaume, and G. Allard. 2011. Diurnal variations of non-structural carbohydrates and nutritive value in alfalfa. Crop Sci. 51:1297-1306.
- Morin, C., G. F. Tremblay, G. Bélanger, A. Bertrand, Y. Castonguay, R. Drapeau, R. Michaud, R. Berthiaume, and G. Allard. 2012a. Nonstructural carbohydrate concentration during field wilting of PM- and AM-cut alfalfa. Agron. J. 104:649-660.
- Morin, C., G. Bélanger, G. F. Tremblay, A. Bertrand, Y. Castonguay, R. Drapeau, R. Michaud, R. Berthiaume, and G. Allard. 2012b. Short communication: Diurnal variations of nonstructural carbohydrates and nutritive value in timothy. Can. J. Plant Sci. 92:883-887.
- Pelletier, S., G. F. Tremblay, G. Bélanger, P. Seguin, R. Drapeau, and G. Allard. 2008a. Delayed harvest affects mineral and NDF concentrations, and digestibility of timothy. Can. J. Anim. Sci. 88:325-329.
- Pelletier, S., R. J. Simpson, R. A. Culvenor, G. Bélanger, G. F. Tremblay, G. Allard, J. Braschkat, and P. J. Randall. 2008b. Dietary cation-anion differences in some pasture species, changes during the season and effects of soil acidity and lime amendment. Australian Journal of Experimental Agriculture, 48:1143-1153.
- Pelletier, S., G. F. Tremblay, C. Lafrenière, A. Bertrand, G. Bélanger, Y. Castonguay, and J. Rowsell. 2009. Nonstructural carbohydrate concentrations in timothy as affected by N fertilization, stage of development, and time of cutting. Agron. J. 101:1372-1380.
- Pageau, and R. Drapeau. 2010a. Forage nonstructural carbohydrates and nutritive value as affected by time of cutting and species. Agron. J. 102:1388-1398.
- Pelletier, S., G. F. Tremblay, A. Bertrand, G. Bélanger, Y. Castonguay, and R. Michaud. 2010b. Drying procedures affect non-structural carbohydrates and other nutritive value attributes in forage samples. Anim. Feed Sci. Technol. 157:139-150.
- Simili da Silva, M., G. F. Tremblay, G. Bélanger, J. Lajeunesse, Y. A. Papadopoulos, S. A. E. Fillmore, and C. C. Jobim. 2012. Energy to protein ratio of grass-legume binary mixtures under frequent clipping. Agron. J. (accepted for publication).

Tremblay, G. F., G. Bélanger, and R. Drapeau. 2005. Nitrogen fertilizer application and developmental stage affect silage quality of timothy (*Phleum pratense* L.). Grass Forage Sci. 60:337–355.

Tremblay, G. F., C. Morin, G. Belanger, A. Bertrand, Y. Castonguay, R. Michaud, and G. Allard. 2010. Non-structural carbohydrate concentrations during wilting of PM- and AM-cut alfalfa. Scientific poster presented at the 2010 ASA-CSSA-SSSA International Annual Meetings, 31 Oct. – 3 Nov., Long Beach, CA. Abstract 293-11.

