Red Clover Improves the Energy to Protein Balance of Lucerne-Grass Herbage

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Abstract

Low ratio of readily fermentable carbohydrate to soluble protein concentrations in lucerne (*Medicago sativa* L.) leads to inefficient use of herbage N by ruminants. To improve the energy to protein balance in lucerne-grass herbage, four proportions of lucerne:red clover (*Trifolium pratense* L.) were compared in mixtures with and without grasses: timothy (*Phleum pratense* L.) and tall fescue (*Schedonorus arundinaceus* Schreb. Dumort.) in Quebec (QC, Canada). In the first post-seeding year, red clover proportion averaged (across grasses and four harvests) 0, 37, 59, and 74% in herbage mixtures. Increasing the proportion of red clover caused a slight but significant decrease in herbage total nitrogen (TN) concentration (32 to 31 g kg^{-1} DM) but substantial decreases in non-protein N (PA), rapidly (PB1) and moderately (PB2) degraded protein fractions, and a significant increase in the slowly degraded protein fractions (PB3+PC) (157 to 308 g kg^{-1} TN). With the inclusion of 74% of red clover, the ratio of soluble sugar to crude protein (CP) in herbage increased from 0.25 to 0.36 because of the increase in the soluble sugar concentration (48 to 66 g kg^{-1} DM). The inclusion of red clover in mixture with lucerne improved the energy to CP balance compared to lucerne alone, and caused a linear increase in the herbage *in vitro* neutral detergent fiber digestibility from 568 to 639 g kg^{-1} aNDF with similar herbage dry matter yield (10.3 Mg ha^{-1}).

Introduction

Optimizing N use efficiency (NUE) in the rumen is at the core of dairy farm profitability while minimizing environmental impacts of dairy cattle production, especially when cows are fed mostly herbage. Herbage legume and grass N is found in protein and non-protein N compounds. Excessive degradation of herbage protein in the rumen can lead to reduced NUE by the cow. In situations where energy is limiting, rumen microorganisms will use protein degradation products as a source of energy thus releasing ammonia, which can then be lost after excretion (Kingston and Theodorou 2000). With sufficient energy in the rumen, herbage N compounds are incorporated into microbial proteins, which can then be used by lactating cows therefore reducing the potential for N losses through urea (Fox et al. 2004). Consequently, to optimize ruminal NUE, rumen energy and N concentrations must be properly balanced.

Carbohydrates, the primary source of energy for ruminant, are essentially classified as non-structural and structural. Degradability of starch depends on size and shape of molecules, but soluble sugars are the most readily available carbohydrates (Lanzas et al. 2007). Total nitrogen (TN) is present in several protein fractions which can be categorized as non-protein nitrogen (PA), quickly degraded protein (PB1), intermediately degraded protein (PB2), slowly degraded protein (PB3), and unavailable protein (PC). While it is simple to estimate NUE as soluble sugar/CP ratio (Parsons et al. 2011), CP may be misleading because it includes slowly degraded (PB3) and non available proteins (PC) which are not as good indicators of N efficiency (Fox et al. 2004). Therefore, ratio involving specifically PA+PB1 fractions would better predict the synchrony of readily available carbohydrates and N compounds in rumen.

Lucerne, the most widely grown herbage legume in Canada, has relatively low readily fermentable carbohydrate concentration and high soluble protein concentration. Red clover could be expected to increase the energy to N balance in alfalfa dominated herbage because of his high concentration of soluble sugars (Sousa et al. 2020). In addition, red clover contains polyphenol oxidase (PPO) which reduces protein degradability in the rumen (Hart et al. 2016), thus potentially increases the (soluble sugars+starch)/(PA+PB1) ratio by lowering the proportion of the PA and PB1 fractions. Our objective was to evaluate the potential of improving the energy to protein balance of lucerne-based herbage mixtures through the addition of red clover.

Methods and Study Site

Experimental plots were seeded in 2017 at Saint-Augustin-de-Desmaures, QC, Canada (46 48’N, 71 23’W). A total of 12 treatments were established as the combination of four proportions of lucerne:red clover (100:0,

75:25, 50:50, and 25:75) times three grass treatments (no grass, timothy, and tall fescue). Plots were assigned to a randomized complete block design with four replications. In the seeding year, P and K fertilizers were applied at seeding based on soil test recommendations for lucerne grass mixes. Following the first harvest in the seeding year, 40 kg N ha^{-1} was applied to all plots to stimulate tillering of grasses. No N fertilizer being applied in the post-seeding year.

Here we report results from the first post-seeding year. Herbage was harvested four times at the late-bud stage of lucerne from 0.6×5 m plot areas at a 7-cm height using a flail mower and weighed. Samples of approximately 500 g were weighed fresh, dried, and reweighed to determine DM yields. Dried herbage samples were ground to pass through a 1-mm screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) and preserved for laboratory analysis. Botanical composition of the herbage was determined by pre-harvesting herbage from a permanent quadrat (50×50 cm) in each plot. After sampling, each seeded herbage species and weeds were separated by hand and dried to determine their contribution to yield on a dry matter basis.

All herbage samples were scanned by visible near-infrared reflectance spectroscopy (VNIRS) using a Foss DS6500 monochromator. A calibration and validation set of herbage samples was selected and chemically analysed for ash, crude fat (CF), neutral detergent fiber assayed with a heat stable α-amylase and sodium sulfate (aNDF), neutral detergent insoluble crude protein (NDICP), total N (TN), crude protein (CP=TN×6.25), *in vitro* true digestibility of dry matter (IVTD), *in vitro* NDF digestibility (NDFd), total carbohydrates (TC=1000-CF-CP-ash), structural carbohydrates (SC=aNDF-NDICP), non-structural carbohydrates (TC-SC), and protein fractions (PA, PB1, PB2, and PB3+PC) as described by Simili da Silva et al. (2013). Soluble sugars and starch were analysed by high-performance liquid chromatography following Bertrand et al. (2018). All nutritive attributes were then predicted by VNIRS for all hebage samples and the energy/protein ratios were calculated from predicted values.

Nutritive attributes were weighted for DM yield observed at each harvest as a proportion of the seasonal yield. Data were analysed with the MIXED procedure of SAS (SAS institute, Cary) in a model including block as random effect along with grass, red clover proportion (RC), and grass×RC interaction as main effects. As there was no grass×RC interaction, data from the three grass treatments were averaged. Polynomial contrasts were used to compare proportions of red clover. Only significant effects are discussed with emphasis herein the response to red clover proportions.

Results

Dry matter yield and botanical composition

Red clover proportions in mixtures during the first post-seeding year averaged 0, 37, 59, and 74% among treatments (Table 1). They differed from the seeding proportions but incremental red clover proportions in mixtures were established as indicated by the significant linear response. Herbage yield per cut as well as seasonal herbage yield did not differ among red clover proportions, averaging 2.6 and 10.3 Mg DM ha^{-1}, respectively.

Herbage digestibility

In vitro true digestibility (IVTD) of dry matter increased linearly from 834 to 857 g kg^{-1} DM and neutral detergent fiber digestibility (NDFd) from 568 to 639 g kg^{-1} aNDF with an increase in red clover proportions from 0 to 74% (Table 1).

Carbohydrates

Herbage total and non-structural carbohydrate concentrations increased linearly with red clover proportions from 664 to 679 g kg^{-1} DM and 309 to 343 g kg^{-1} DM, respectively. Soluble sugar concentration increased from 48 to 66 g kg^{-1} DM but in a quadratic manner. In contrast, structural carbohydrate and starch concentrations decreased from 355 to 336 g kg^{-1} DM and 43 to 32 g kg^{-1} DM, respectively, with an increase in red clover proportions.

Proteins

All nutritive attributes related to proteins (i.e., TN, PA, PB1, PB2, and PB3+PC) were significantly affected by an increase of the red clover proportion in lucerne-based herbage mixtures (Table 1). Concentrations of PA, PB1 and PB2 decreased with an increase in red clover proportion; this decrease ranged from 284 to 234 g kg^{-1} TN for PA, 155 to 111 g kg^{-1} TN for PB1, and 404 to 348 g kg^{-1} TN for PB2. On the other hand, the PB3+PC concentration increased from 157 to 308 g kg^{-1} TN with an increasing proportion of red clover.

Table 1. Effects of increasing the red clover proportion (RC) in lucerne-based mixtures on herbage yield and nutritive attributes in a first post-seeding year at Saint-Augustin-de-Desmaures, QC (Canada)

	Red clover at seeding (RC, %)					*P*-value		
Variables	**0**	**25**	**50**	**75**	**SEM**	**RC**	**Lin**	**Quad**
Lucerne proportion (%)	90	58	34	21	3	<0.01	<0.01	0.60
Red clover proportion (%)	0	37	59	74	3	<0.01	<0.01	0.93
Yield per cut (Mg DM ha^{-1})	2.6	2.6	2.5	2.6	0.1	0.85		
Seasonal yield (Mg DM ha^{-1})	10.4	10.5	10.1	10.2	0.4	0.85		
Digestibility								
IVTD (g kg^{-1} DM)	834	844	850	857	2	<0.01	<0.01	0.48
NDFd (g kg^{-1} aNDF)	568	616	626	639	5	<0.01	<0.01	0.05
Carbohydrates (g kg^{-1} DM)								
Total	664	674	675	679	2	<0.01	<0.01	0.44
Structural	355	348	339	336	4	<0.01	<0.01	0.62
Non-structural	309	326	337	343	4	<0.01	<0.01	0.99
aNDF	389	398	394	397	3	0.08		
Soluble sugars (SS)	48	53	60	66	1	<0.01	<0.01	0.02
Starch	43	35	33	32	1	<0.01	<0.01	0.10
Proteins								
Total N (TN, g kg^{-1} DM)	32.3	32.0	31.5	30.7	0.3	<0.01	<0.01	0.08
PA (g kg^{-1} TN)	284	261	244	234	3	<0.01	<0.01	0.63
PB1 (g kg^{-1} TN)	155	134	117	111	4	<0.01	<0.01	0.97
PB2 (g kg^{-1} TN)	404	368	358	348	5	<0.01	<0.01	0.29
PB3+PC (g kg^{-1} TN)	157	237	281	308	6	<0.01	<0.01	0.48
Ratios								
SS/Crude protein	0.25	0.28	0.32	0.36	0.01	<0.01	<0.01	0.02
(SS+Starch)/(PA+PB1)	1.1	1.2	1.4	1.5	0.1	<0.01	<0.01	0.03

Carbohydrate/protein ratios

An increase in all energy/protein ratios was observed as the proportion of red clover in the herbage mixture increased, but more importantly for RC proportions greater than 50% (Table 1). The soluble sugars/CP and (soluble sugars+starch)/(PA+PB1) ratios increased from 0.25 to 0.36, and 1.1 to 1.5, respectively.

Conclusions/Implications

Past studies have suggested that an increase in herbage carbohydrate to protein ratio can potentially improve N utilization by rumen bacteria and hence increase N use efficiency (Parsons et al. 2011). Our hypothesis was that this ratio could be improved by the inclusion of red clover in lucerne-based mixtures, due to its greater concentration of soluble sugars and lower protein degradability when compared to lucerne.

Our results confirm that increasing the red clover proportion increased the soluble sugar concentration along with the non-structural carbohydrate fraction in lucerne-based mixtures. However, the increase in the red clover proportion reduced the starch concentration in the mixture. Sousa et al. (2011) also observed a greater concentration of soluble sugars in red clover than lucerne (62.2 vs 36.7 g kg^{-1} DM).

Increasing the proportion of red clover caused a slight but significant decrease in herbage total nitrogen (TN) concentration, substantial decreases in non-protein N and rapidly and moderately degraded protein fractions, and a significant increase in the proportion of slowly degraded and unavailable protein fractions. Similar results were obtained by Sousa et al. (2020). This change in protein fractions strongly suggests that PPO presents in red clover did lower protein degradability in the herbage mixture. Past studies have already reported the action of PPO in red clover and its role in reducing protein degradability (Hart et al. 2016).

The addition of red clover in lucerne-grass herbage mixtures significantly improved their energy to protein ratios in the first post-seeding year, thus confirming our hypothesis. In addition, dry matter and NDF digestibilities increased linearly with the addition of red clover in the mixture with no negative impact on herbage yield during the first post-seeding year. Adding red clover to lucerne-based herbage mixtures could be an approach to increase N use efficiency in dairy cattle by increasing soluble sugars and reducing protein degradability with the added benefits of improving herbage digestibility and not affecting herbage yield.

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