UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

VALOR NUTRICIONAL DA MASSA DE FORRAGEM DE LEGUMINOSAS E GRAMÍNEAS EM CONSORCIAÇÃO

Autor: Michele Simili da Silva
Orientador: Prof. Dr. Clóves Cabreira Jobim
Coorientadores: Dr.Gaetan Tremblay
Dr.Gilles Bélanger

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Tese apresentada, como parte das exigências para obtenção do título de DOUTOR EM ZOOTECNIA, no Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá - Área de Pastagens e Forragicultura

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TITULAÇÃO: Doutora em Zootecnia - Área de Concentração Pastagem e Forragicultura

APROVADA em 24 de setembro de 2012.

Prof. Dr. Ulysses Cecato

Profª Drª Lúcia Maria Zeoula

Prof. Dr. Ricardo Andrade Reis

Prof. Dr. Aníbal de Moraes

Prof. Dr. Clóves Cabreira Jobim
(Orientador)

"Quando me amei de verdade, compreendi que em qualquer circunstância, eu estava no lugar certo, na hora certa, no momento exato.
E então, pude relaxar.
Hoje sei que isso tem nome... AUTO-ESTIMA.
Quando me amei de verdade, pude perceber que minha angústia, meu sofrimento emocional, não passa de um sinal de que estou indo contra minhas verdades.
Hoje sei que isso é...AUTENTICIDADE.
Quando me amei de verdade, parei de desejar que a minha vida fosse diferente e comecei a ver que tudo o que acontece contribui para o meu crescimento.
Hoje chamo isso de... AMADURECIMENTO.
Quando me amei de verdade, comecei a perceber como é ofensivo tentar forçar alguma situação ou alguém apenas para realizar aquilo que desejo, mesmo sabendo que não é o momento ou a pessoa não está preparada, inclusive eu mesmo.
Hoje sei que o nome disso é... RESPEITO.
Quando me amei de verdade comecei a me livrar de tudo que não fosse saudável... Pessoas, tarefas, tudo e qualquer coisa que me pusesse para baixo. De início minha razão chamou essa atitude de egoísmo.
Hoje sei que se chama... AMOR PRÓPRIO.
Quando me amei de verdade, deixei de temer o meu tempo livre e desisti de fazer grandes planos, abandonei os projetos megalômanos de futuro.
Hoje faço o que acho certo, o que gosto, quando quero e no meu próprio ritmo.
Hoje sei que isso é... SIMPLICIDADE.
Quando me amei de verdade, desisti de querer sempre ter razão e, com isso, errei muitas vezes menos.
Hoje descobri a... HUMILDADE.
Quando me amei de verdade, desisti de ficar revivendo o passado e de preocupar com o futuro. Agora, me mantenho no presente, que é onde a vida acontece.
Hoje vivo um dia de cada vez. Isso é... PLENITUDE.
Quando me amei de verdade, percebi que minha mente pode me atormentar e me decepcionar. Mas quando a coloco a serviço do meu coração, ela se torna uma grande e valiosa aliada.
Tudo isso é... SABER VIVER!!!"

Charles Chaplin

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BIOGRAFIA

Michele Simili da Silva, filha de Cidinei Rodrigues da Silva e Isaura Simili da Silva, nasceu em Jundiaí, São Paulo, no dia 2 de setembro de 1982.

Em agosto de 2001, ingressou na Universidade Federal de Lavras, e em junho de 2006, obteve o título de Zootecnista.

Em março de 2007, iniciou no mestrado em Zootecnia, na Universidade Estadual de Maringá, e em abril de 2009, obteve o título de Mestre em Zootecnia na área de Pastagens e Forragicultura.

Em março de 2009, ingressou no doutorado em Zootecnia, na Universidade Estadual de Maringá e em maio de 2011, iniciou o estágio de doutorado no Agriculture and Agri-Food, Soils and Crops Canada.

No dia 24 de outubro de 2012, submeteu-se à banca para defesa da Tese para obtenção do título de Doutora em Zootecnia na área de Pastagens e Forragicultura.

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LISTA DE ABREVIATURAS

ADF: acid detergent fiber

ADL: acid detergent lignin

aNDF: neutral detergent fiber assayed with a heat stable α-amylase

CP: crude protein

CNCPS: Cornell Net Carbohydrate and Protein System

DM: dry matter

dNDF: *in vitro* digestibility of NDF

IVTD: *in vitro* true digestibility of DM

Kb: Kentucky bluegrass

Mf: meadow fescue

Mb: meadow bromegrass

aNDF: neutral detergent fiber with α-amilase

NIRS: Near Infrared Reflectance Spectroscopy

O: orchardgrass

PA: protein fraction A

PCA: principal component analysis

Rc, reed canarygrass

RPD: ratio of standard error of prediction to standard deviation

SC: structural carbohydrates

SEP(C): standard error of prediction corrected for bias

SD: standard deviation

Tf: tall fescue

Ti: timothy

TN: total nitrogen

TC: total carbohydrates

WSC: water soluble carbohydrates

RESUMO

O objetivo deste trabalho foi avaliar o potencial de misturas, simples e complexas, entre leguminosas e gramíneas por meio de medidas de produção de massa de forragem, da composição nutricional e do balanço entre energia prontamente disponível e proteína. Foram conduzidos dois experimentos em duas localidades Lévis e Normandin, QC-Canadá. O objetivo do primeiro experimento foi determinar a melhor combinação entre misturas de uma espécie de gramínea com uma espécie de leguminosa. Seis espécies de gramíneas, timothy (*Phleum pratense* L.), Kentucky bluegrass (*Poa pratensis* L.), tall fescue (*Festuca arundinacea* Schreb.), orchardgrass (*Dactylis glomerata* L.), meadow bromegrass (*Bromus biebersteinii* Roemer & J.A. Schultes), e meadow fescue (*Festuca elatior* L.) foram semeadas com cada uma das três espécies de leguminosas alfafa (*Medicago Sativa* L.), trevo branco (*Trifolium repens* L.) e cornichão (*Lotus corniculatus* L.) em dois sítios. Foi determinado a produção de massa de forragem e as frações de carboidratos e proteínas, juntamente com outros atributos de valor nutricional nas duas primeiras colheitas do primeiro ano de produção. As espécies de gramíneas e leguminosas influenciaram significativamente a maioria das variáveis avaliadas. Misturas com alfafa apresentaram maior relação de carboidratos solúveis e proteína bruta (CS/PB), assim como maior relação da soma das frações de carboidrato A+B1e a soma das frações de proteína A+B1. Porém misturas com alfafa também apresentaram as maiores concentrações de FDA e FDN, a mais baixa digestibilidade da FDN e a mais baixa digestibilidade verdadeira que a média das três leguminosas, com similar produção de MS. Misturas com *Festuca elatior* e *Festuca arundinacea* tiveram as maiores taxas de CS/PB, produção de MS e concentrações de FDA e FDN que a média das seis espécies de gramíneas. Misturas de *Festuca elatior* com qualquer uma das três espécies de leguminosas tiveram a melhor combinação de altas taxas de CS/PB e produção de MS. A mistura de alfafa com *Festuca elatior* mostrou alta relação CS/PB (0.70), e a maior produção de MS, porém com digestibilidade abaixo da média. O objetivo do segundo experimento foi comparar quatro misturas de espécies de gramíneas combinadas com duas espécies de leguminosas (alfafa *Medicago sativa* L. cv. CRS1001) ou Cornichão *Lotus corniculatus* L. cv. AC Langille). As quatro misturas de gramíneas foram: 1) timóteo, *Festuca elatior*, falaris and *Poa pratensis* ; 2) *Poa pratensis* , *Festuca elatior*, Dactylis e Bromus ; 3) *Festuca elatior*, timóteo and *Poa pratensis* ; 4) Falaris, *Poa pratensis* Kentucky bluegrass, *Festuca arundinacea* and Bromus. As oito misturas de forragens foram semeadas em dois sítios experimentais, em que as leguminosas formavam as parcelas principais e as misturas de gramíneas as subparcelas. As misturas baseadas em alfalfa tiveram as maiores concentrações de FDA, FDN e carboidratos totais, maiores taxas de CS/PB e (CA+CB1)/(PA+PB1) e as mais baixas concentrações de nitrogenio total e fração A da proteína (PA) que a média das oito misturas complexas. As misturas com *Festuca elatior* apresentaram maior produção de MS comparada a média das oito misturas complexas. O mix de espécies de gramínea *Festuca elatior*, timóteo e *Poa pratensis* (MfTiKb) teve a mais baixa concentração de FDA e as maiores dNDF and IVTD, enquanto o mix timóteo, *Festuca elatior*, falaris e *Poa pratensis* (TiMfRcKb) teve as maiores taxas de CS/PB e CB1+CA/PB1+PA que a média das oito misturas complexas. A mistura complexa de alfafa com timóteo, *Festuca elatior* , falaris e *Poa pratensis* (ATiMfRcKb) teve a maior taxa de CS/PB. As misturas complexas com alfafa e meadow fescue tiveram as melhores taxas de CS/PB e as maiores produções de MS. Misturas de *Festuca elatior* com alfafa, trevo branco ou cornichão geralmente providenciaram a melhor combinação de balanço energia proteína, produção de MS e digestibilidade. A mistura de alfafa com *Festuca elatior* apresentou o melhor balanço energia prontamente disponível e proteína e a melhor produção de MS, no entanto apresentou baixa digestibilidade. O mix de espécies de gramínea composto por timóteo, *Festuca elatior* , falaris e *Poa pratensis* (TiMfRcKb) providenciou a combinação da taxa mais alta do balanço entre energia prontamente disponível e proteína e alta produção de MS. Complexas misturas com alfafa e *Festuca elatior* tiveram a melhor taxa energia/proteína e melhor produção de MS. Os resultados das duas primeiras colheitas da produção do primeiro ano providenciaram usuais e originais informações sobre a composição desejada das espécies das misturas, que combinam relação entre energia e proteína e produtividade de MS.

Palavras chave: associação gramínea-leguminosa, forrageiras temperadas, massa de forragem, qualidade da forragem

ABSTRACT

The objective of this study was to evaluate the potential of mixtures, simple and complex, between legumes and grasses by production measurements of herbage mass, nutritional composition and balance between readily-available energy and protein. Two experiments were carried out at two locations Lévis and Normandin. The objective of the first experiment was todetermine the best combination of a single grass species and one of the legume species. Six grass species, timothy (*Phleum pratense* L.), Kentucky bluegrass (*Poa pratensis* L.), tall fescue (*Festuca arundinacea* Schreb.), orchardgrass (*Dactylis glomerata* L.), meadow bromegrass (*Bromus biebersteinii* Roemer & J.A. Schultes), and meadow fescue (*Festuca elatior* L.) were seeded with one of three legume species, alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.), and birdsfoot trefoil (*Lotus corniculatus* L.) at two sites. Carbohydrate and protein fractions using the Cornell net carbohydrate and protein system along with other nutritive value attributes and dry matter (DM) yield were determined at the two first harvests of the first production year. Grass and legume species in the mixture significantly affected most attributes. Alfalfa mixtures had greater ratios of water soluble carbohydrates (WSC) to crude protein (CP) and carbohydrate fractions A+B1 to protein fractions A+B1 also had greater acid detergent fiber (ADF) and neutral detergent fiber (aNDF) concentrations, and lower *in vitro* neutral detergent fiber digestibility (dNDF), and *in vitro* true DM digestibility (IVTD) than the average of the three legume species with similar DM yield. Mixtures with meadow fescue and tall fescue had greater WSC/CP ratio, DM yield, and ADF and NDF concentrations than the average of the six grass species. Mixtures of meadow fescue with any of the three legume species generally had the best combination of high ratios of WSC/CP and DM yield. The alfalfa and meadow fescue mixture had numerically the best WSC/CP ratio (0.70), the highest DM yield but low average IVTD. The objective of the second experiment was compare four grass mixtures combined with two forage legumes (grazing type alfalfa, A, *Medicago sativa* L. cv. CRS1001) or birdsfoot trefoil, B, *Lotus corniculatus* L. cv. AC Langille). The grass mixtures used were: 1)timothy, meadow fescue, reed canarygrass and Kentucky bleugrass; 2)Kentucky bluegrass, tall fescue, orchardgrass and meadow brome; 3) meadow fescue, timothy and Kentucky bluegrass; 4) reed canarygrass, Kentucky bluegrass, tall fescue and Meadow brome. The eight forage mixtures were seeded at two sites with legume species as main plots and grass species mix as subplots. Data were averaged across the two sites and the two first harvests of the first production year and analysed by ANOVA followed by a principal component analysis. Alfalfa-based mixtures had greater ADF, NDF, and TC concentrations, greater WSC/CP and (CA+CB1)/(PA+PB1) ratios, and lower concentrations of TN and PA than the average of the eight complex mixtures. The grass species mixes with meadow fescue had the greater DM yield compared to the average of the eight complex mixtures. The MfTiKb grass species mix had a lower ADF concentration and greater dNDF and IVTD, whereas the TiMfRcKb grass species mix had greater WSC/CP and CB1+CA/PB1+PA ratios than the average of the eight complex mixtures. The ATiMfRcKb complex mixture had the greatest WSC/CP ratio. The complex mixtures with alfalfa and meadow fescue had the best energy/protein ratio and DM yield. Mixtures of meadow fescue with alfalfa, white clover, or birdsfoot trefoil generally provided the best combination of a high energy/protein ratio, high DM yield, and good digestibility. The mixture of alfalfa with meadow fescue had the best energy/protein balance and DM yield but a low average digestibility. The TiMfRcKb grass species mix provided the combination of high readily-available energy to protein balance and high DM yield. The complex mixtures with alfalfa and meadow fescue had the best readily-available energy to protein ratio and DM yield. These results from the first two harvests of the first production year provide useful and novel information on the desired species composition of binary mixtures that combine energy to protein ratio and DM yield.

Key words: forage mass, forage quality, legume-grass mixture, temperate forages

1. INTRODUCÃO GERAL

Muitos produtores preferem trabalhar com pastagens de gramíneas em monocultura por serem mais fáceis de manejar (Clark, 2001). Entretanto, de acordo com Tracy e Sanderson (2004), pastagens em monocultura são mais vulneráveis ao estresse ambiental do que em consórcio. Entre alguns dos benefícios da consorciação entre leguminosas e gramíneas está o potencial em disponibilizar, com mais consistência, massa de forragem em uma vasta gama de ambientes em comparação a gramíneas em monocultura (Haynes, 1980).

Uma consorciação botanicamente estável e produtiva entre gramíneas e leguminosas é frequentemente difícil de manter por causa do alto grau de competição entre os seus componentes. Gramíneas e leguminosas podem competir por irradiação, água e minerais do solo quando cultivadas em consórcio (Jones et al., 1988). Uma composição botânica estável permite produção máxima durante os períodos de pico de crescimento de cada espécie, sem exclusão competitiva de qualquer dos componentes (Camlin, 1981).

A concentração dos nutrientes na forragem é afetada pelo estádio de crescimento e pela espécie forrageira. Em pastagens consorciadas é importante a escolha de uma leguminosa mais persistente, que seja compatível com as gramíneas introduzidas, e forneça N biologicamente fixado que possa aumentar o rendimento do pasto mantendo alta qualidade da forragem (Zemenchik et al., 2001).

Em situações de pastejo, normalmente falta fornecimento adequado de energia para os microrganismos do rúmen, em relação a grande quantidade de N disponível na dieta, especialmente em animais de alta produção. A assincronia na disponibilidade de energia e proteína pode resultar em grandes quantidades de N a ser perdido como amônia, cuja maioria é excretada na forma de ureia na urina (Ellis et al., 2011). O uso de gramíneas com maiores teores de açúcares pode corrigir este desequilíbrio de carbono e N, promovendo melhorias na eficiência de utilização da proteína dietética com melhor eficiência microbiana ruminal (Miller et al., 2001).

É esperado das gramíneas que estas forneçam a maior parte da energia para o gado. Enquanto o papel das leguminosas, nas misturas, é suplementar o N das gramíneas e melhorar o conteúdo nutricional da massa de forragem, particularmente em proteína, fósforo e cálcio (Fageria et al., 2010).

A produção de massa de forragem de alto valor alimentício é importante para reduzir o impacto dos sistemas de produção da pecuária sobre o meio ambiente. Nesse contexto, pesquisas têm incidido sobre a determinação da digestibilidade, energia e valor proteico da forragem produzida em sistemas de cultivo singular ou em misturas de gramíneas e leguminosas (Huhtanen, 2011).

2. REVISÃO DE LITERATURA

2.1. Gramíneas e leguminosas em consorciação

Leguminosas forrageiras possuem geralmente teores mais elevados de proteína bruta, pectina, lignina e minerais do que gramíneas e mais baixos teores de celulose, hemicelulose e carboidratos solúveis em água. Em contraste com essas vantagens, a falta de persistência e potenciais problemas com componentes antinutricionais são desvantagens mesmo em consórcio com gramíneas (Laidlaw e Teuber, 2001).

Em muitas situações os produtores usam misturas de gramíneas e leguminosas, em vez de monoculturas agrícolas para aumentar a produção total de forragem e para produzir uma forragem mais equilibrada nutricionalmente para a alimentação do gado (Giambalvo et al., 2011). Os rendimentos são geralmente mais elevados em misturas por causa da utilização mais eficiente de luz, água e nutrientes (Corre-Hello et al., 2006) e transferência de nitrogênio fixado simbioticamente para as gramíneas.

Uma vantagem no rendimento em misturas de espécies pode ocorrer quando as culturas diferem na utilização de recursos para crescimento, de tal forma que, quando elas são cultivadas em conjunto são capazes de complementar uma a outra e assim, fazer melhor uso dos recursos do que quando cultivadas separadamente. O uso mais eficiente de recursos limitados em consórcio podem ocorrer se as culturas associadas usarem os recursos, quer em tempos diferentes e ou em diferentes partes do perfil do solo ou do dossel aéreo (Willey, 1979).

As leguminosas podem proporcionar aumentos na produção e melhorias na qualidade da massa de forragem do consórcio (Sleugh et al., 2000; Albayrak et al., 2011), aumentando a concentração de proteínas e a digestibilidade por causa da redução na concentração de fibras (Fraser e Kunelius, 1995). Gramíneas normalmente possuem mais fibras que leguminosas, especialmente nas folhas. No entanto, a fração fibra das gramíneas é mais digestíveis que as leguminosas. Dependendo da maturidade da forrageira, ruminantes digerem 40-50% da fibra de leguminosas e 60-70% da fibra de gramíneas temperadas (Buxton et al., 1995).

É geralmente aceito que gramíneas normalmente tenham uma competitiva vantagem sobre as leguminosas, e portanto, tendem a dominar as pastagens. No entanto, para manter a produtividade da pastagem alta, é desejável o equilíbrio entre gramíneas e leguminosas (Haynes, 1980).

A compatibilidade entre gramíneas e leguminosas depende de suas características morfológicas e fisiológicas, em combinação com a resposta de cada uma ao manejo, clima e solo (Frame et al., 1998). Para otimizar a produção e qualidade da forragem, o manejo de corte de uma consorciação de leguminosa com gramínea deve ser baseado no calendário de colheita da leguminosa. Desta forma, o manejo de corte da leguminosa influencia na escolha da gramínea (Tesar e Marble, 1988).

2.1.2 Caracterização das espécies de leguminosas

2.1.2.1 Trevo Branco (*Trifolium repens*)

O trevo branco é uma das mais importantes espécies de trevo para consorciação com gramíneas dentro do gênero *Trifolium*. Este gênero, compreendendo cerca de 240 espécies, é encontrado em regiões temperadas úmidas, áreas mediterrâneas e algumas partes frias subtropicais do mundo. Possui uma gama de adaptação climática vasta e alta qualidade nutricional. Entretanto apenas algumas espécies se destacam em sistemas de pastagens. (Frame et al., 1998).

O trevo branco é persistente em sistemas de pastagens, podendo persistir por ressemeadura natural, e quando é severamente prejudicado pelo inverno, pode persistir através do enraizamento de estolões de extremidades jovens, que estão mais aptos a sobreviver durante o inverno que as partes mais velhas da planta. O trevo branco é considerado uma forrageira perene, porém morfologicamente e fisiologicamente as várias partes que compõem a planta geralmente são capazes de sobreviver apenas dois anos. Na maioria das condições estolões produzidos durante o ano anterior, morrem no final da primavera ou início do verão do próximo ano. Assim, com efeito, as várias porções da planta podem ser consideradas como tendo um hábito bienal (Smith, 1981).

A massa de forragem do trevo branco fornece alto teor de proteína, alta suculência, alto conteúdo de minerais e baixa fibra. A porcentagem de proteína pode variar de 20 a 30% na MS e raramente cair abaixo de 18 a 20% (Smith, 1981). Entretanto, esta leguminosa pode causar desordens digestivas em ruminantes se não pastejada cuidadosamente.

Diferentes espécies e cultivares de gramíneas têm contrastante efeito competitivo na performance do trevo branco em pastagens consorciadas. Existem algumas espécies de gramíneas, como a festuca, que são menos competitivas e portanto altamente compatíveis, e outras espécies como o *Dactilys* que são altamente agressivas (Frame et al., 1998). Os fatores que influenciam o balanço de gramíneas e trevos em pastagens consorciadas são consideravelmente mais complexos que aqueles manejados quando misturas de somente espécies ou cultivares de gramíneas estão envolvidos.

O balanço gramínea-trevo é muito susceptível as mudanças no manejo e no ambiente, especialmente a respeito dos níveis de adubação de nitrogênio aplicados. Em pastagens de gramínea em consorciação com trevo, em que o N não é aplicado ou é aplicado apenas em um baixo nível o trevo tem uma vantagem, mas em altos níveis de adubação de nitrogênio as gramíneas são favorecidas (Camlin, 1981).

Em revisão de alguns trabalhos envolvendo consorciação de gramíneas com trevo branco, Chestnutt e Lowe (1970) concluíram que em geral entre as gramíneas comuns, o azevém perene (*Lolium perenne*) e a festuca (*Festuca pratensis*) foram as mais compatíveis com trevo branco. Enquanto o Dactilys foi a menos compatível e a *Festuca arundinacea* e o capim timóteo foram intermediárias.

Na Região sul do Brasil ocorre ambiente propício a utilização de algumas leguminosas temperadas de alto valor nutricional como os trevos, e as principais utilizações das espécies deste gênero são em consorciação com gramíneas estivais ou hibernais. As leguminosas também podem ser utilizadas em sobressemeadura sobre as pastagens nativas e para produção de feno ou silagem. Dentre as especies do gênero *Trifolium* o trevo branco se destaca pelos seus altos rendimentos e elevado valor nutritivo (Dall'Agnol e Scheffer-Basso, 2004). No Rio grande do Sul, o trevo branco é uma das espécies de leguminosas mais usadas em pastagens consorciadas, para a utilização direta em pastejo durante o inverno e primavera, podendo vegetar o ano todo em regiões mais frias e com boa distribuição de chuvas (Paim & Riboldi, 1994). No planalto catarinense, as cultivares mais recomendadas são Landino Regal, Jacuí S2 e Bayucuá (Dall'Agnol & Scheffer-Basso, 2004).

2.1.2.2- Cornichão (*Lotus corniculatus* L.)

O cornichão é cultivado comercialmente em mais de 20 países, nas Ilhas Dritsh, ao longo de grande parte da Europa, em alguns países da América do Sul (Argentina, Brasil, Chile e Uruguai) e em algumas partes da Índia, Austrália e nova Zelândia. No Canadá esta leguminosa é cultivada principalmente nas províncias orientais (Beuselinck e Grant, 1995).

O cornichão é cultivado nos estados do sul no Brasil, onde o clima subtropical é mais apropriado para seu desenvolvimento. Desde a sua introdução no Rio Grande do Sul na década de 1960, a única cultivar comercial disponível é a São Gabriel, cujo hábito de crescimento ereto faz a sua sobrevivência sob pastejo difícil (Scheffer-Basso et al., 2011).

Esta legumininosa é capaz de melhorar a disponibilidade de proteína na dieta animal e suplementar nitrogênio para plantas por fixação simbiótica. O cornichão é frequentemente empregado em misturas porque esta leguminosa é adaptada a uma ampla gama de ambientes, conseguindo crescer bem em solos ácidos e tolerar frequentes condições de alagamento (McKenzie et al., 2004). É adaptada a pastagens permanentes e de baixa manutenção, possui uma vida útil de 5-6 anos em pastagens naturais e até 8 ou 9 anos em pastagens semeadas (Strelkov, 1980).

O cornichão não causa problemas de timpanismo, talvez por conter tanino, que precipita as proteínas solúveis e as tornam incapazes de produzir espumas estáveis no rúmen. Também, as paredes celulares do cornichão se rompe mais lentamente do que as de alfafa ou trevo, podendo permitir a liberação mais lenta das substâncias causadoras de timpanismo (Lees et al., 1981).

De acordo com Smith (1981), o cornichão pode persistir sobre pastejo contínuo e rende melhor do que alfalfa ou trevo vermelho, mesmo mantendo um baixo nível de reservas de raiz de carboidratos durante o período vegetativo, em virtude de seu hábito de crescimento. Os brotos de alfafa e trevo vermelho crescem quase na vertical, enquanto os brotos do cornichão são basicamente prostrados. Deste modo, cortes frequentes e rentes remove quase toda a área de folha de alfafa e trevo vermelho, mas muitas folhas permanecem no restolho prostrado do cornichão.

2.1.2.3 Alfafa (*Medicago sativa*)

Alfafa é uma leguminosa herbácia perene que pode sobreviver a temperaturas abaixo de -25 ºC e acima de 50ºC. Esta leguminosa é altamente tolerante a seca e produz mais proteínas por hectare do que alguns grãos e oleaginosas. O número de colheitas por ano é dependente do estádio de maturidade durante a colheita e das condições ambientais (Barnes e Sheaffer, 1995). A maturidade na colheita é o principal fator limitante na produção e qualidade da forragem. Maiores injúrias ocorrem quando cortes são praticados durante períodos de mínima reserva na raiz. Cortes adiantados proporcionam maior qualidade, porém repetidos cortes em estádios imaturos reduzem o vigor, a produção e a longevidade do stand (Probst e Smith, 2011).

Em resposta ao clima frio e ao encurtamento dos dias durante o outono as cultivares de alfalfa se tornam dormentes durante o inverno rigoroso. A reação de dormência envolve complexas mudancas fisiológicas por parte da planta em preparação para o inverno e resulta em um decréscimo no crescimento da forragem e um aumento no estoque de carboidratos de reserva (Mckenzie et al., 1988).

Em consorciação, o competitivo balanço entre alfalfa e gramíneas é fortemente influenciado pelo manejo e meio ambiente. A consorciação de alfafa com gramíneas, entre outros benefícios, pode ajudar a mitigar os riscos de erosão uma vez que as fibras da raiz das gramíneas resistem a erosão do solo melhor que a alfalfa (Tesar e Marble, 1988). Além de diminuir os riscos de empanzinamento de animais em pastejo.

Na alfafa as saponinas são consideradas as principais substâncias antinutricionais encontradas causadoras de timpanismo, sendo que 16% da variação total de saponinas na alfafa é atribuída a diferenças entre cultivares (Hanson et al., 1973). O uso de cultivares de alfafa com teor de taninos suficiente para diminuir a solubilidade da proteína (Weimer, 1999), também podem auxiliar a evitar e a contornar o problema de timpanismo em ruminantes.

Apesar de ser uma das forrageiras mais difundidas em países de clima temperado, recentemente a alfafa é cultivada com sucesso em ambientes tropicais. No Brasil, até 1968, o Estado do Rio Grande do Sul respondia por mais de 70% da área cultivada com alfafa. Atualmente, verifica-se aumento da área plantada com alfafa nas regiões Sudeste e Centro-Oeste, em função da crescente implantação de sistemas intensivos de produção com bovinos de leite. No entanto, a expansão do uso dessa cultura depende de alguns fatores, que vão desde a escolha da cultivar mais adaptada à região até a adoção de práticas agrícolas que permitam seu estabelecimento e sua persistência, aumentem a produção e melhorem a qualidade da forragem (Rassini et al., 2007).

2.1.3 Caracterização das espécies de gramínea de clima temperado

As condições climáticas do Brasil favorecem mais o cultivo de gramíneas tropicais do que gramíneas temperadas. Durante o verão, condições favoráveis de temperatura, disponibilidade de água e radiação, garantem elevados índices produtivos de gramíneas tropicais. No entanto, com a chegada do outono e inverno, a queda da temperatura, a escassez de chuvas e a baixa luminosidade limitam o crescimento vegetal, gerando um déficit entre a oferta e a demanda de massa de forragem (Simili, 2012). Nesta fase de baixa produção a sobressemeadura de forrageiras de inverno em pastagens formadas com espécies perenes de clima tropical passa a ser uma opção considerável. No sul do Brasil, as espécies de gramíneas de clima temperado mais utilizadas são as anuais, especialmente a aveia e o azevém.

Dentre as espécies temperadas perenes já submetidas ao melhoramento genético e introduzidas no Brasil apenas a festuca tem sido cultivada para a formação de pastagens (Rosa et al., 2008). O uso de pastagens perenes como Timóteo, Kentucky bluegrass, Festucas, Capim dos pomares, Bromus e Falaris contribuem para o desenvolvimento de sistemas de produção que reduzem as práticas de preparo de solos, podem recuperar nutrientes em maiores profundidades que os cultivos com pastagens anuais, além de restaurar o fluxo de matéria orgânica do solo (Dubeux Júnior et al., 2006).

De acordo com McElroy e Kunelius (1995), o capim timóteo (*Pheum pratense*) é uma gramínea amplamente adaptada a ambientes temperados e úmidos e não persiste em condições de seca. Não possui rizomas e não é uma gramínea agressiva. No Canadá esta é uma gramínea forrageira importante nas províncias do Atlântico, Québec e Ontário. É bem adaptada para as condições do norte da Europa e é também produzida nas áreas temperadas da América do sul, Austrália e norte do Japão. O campim timóteo é uma das gramíneas de estação fria mais tolerante ao frio, sua rebrota depois da desfoliação é variável e usualmente mais devagar que a maioria das outras espécies de gramíneas (Kunelius et al., 2006).

A *Poa pratensis*, conhecida como Kentucky bluegrass, é uma espécie naturalizada que é comumente encontrada em pastagens no leste do Canadá e em outras regiões temperadas (Durr, 2005). É uma gramínea de baixa para média altura, de longa vida, altamente palatável que possui folha lisa e macia, de verde para verde escuro com pontas em forma de barco. Se fixa através de rizomas para formar um gramado denso e cresce melhor em clima frio e úmido sobre solos férteis e bem-drenados, com pH entre 6 e 7. A produtividade do kentucky bluegrass é aumentada substancialmente com adequada rotação de pastagem e descanso. Devido a sua larga proporção de folhas próximas a superfície do solo e abaixo da altura de pastejo em pastagens manejadas esta gramínea é mais tolerante ao sobre pastejo que a maioria das outras gramíneas de estação fria (Hall, 1996).

A *Schedonorus phoenix* (Scop.) Holub, internacionalmente conhecida como tall fescue, é cultivada na maior parte da Europa, porém outros países incluindo Japão, sul do Canadá, Austrália, Nova Zelândia, México, Colômbia, Argentina, partes da África e EUA também tem cultivado essa gramínea com sucesso (Sleper e West, 1996). A “tall fescue” e a Festuca pratensis, conhecida como “meadow fescue” são similares morfologicamente, e são difíceis de distinguir. No entanto, entre outras características a tall fescue é um pouco mais robusta que a meadow fescue, e possui pelos sobre as suas aurículas (Smith, 1981). Tall fescue é uma gramínea com enraizamento profundo, que tem a habilidade de se espalhar e formar um gramado denso por meio de rizomas curtos (Dale smith, 1981). É comumente usada para pasto, feno, silagem e conservação do solo. É uma gramínea agressiva e possui uma grande adaptabilidade a várias condições ambientais, incluindo solos ácidos e inférteis, propensos a seca e pobremente drenados. Também são tolerantes ao calor, ao superpastejo, e a manejos inadequados (Casler, 2007).

A festuca tall fescue é flexível quanto a utilização para produção de forragem e complementa o uso de outras espécies forrageiras. Esta é frequentemente utilizada em misturas com leguminosas como trevo branco, trevo vermelho e cornichão, especialmente na primavera até o início do verão (Sleper e West, 1996).

O capim dos pomares (*Dactylis glomerata* L.) é uma gramínea perene de estação fria, de alto crescimento cultivada em todos os continentes. Esta gramínea é usada principalmente para pastagens em associação com trevo branco ou alfafa, sendo mais amplamente cultivada com trevo branco. Tem seu crescimento mais cedo na primavera do que a leguminosa e se recupera rapidamente após desfoliação (Smith, 1981). O capim dos pomares é considerado uma das mais responsivas gramíneas de estação fria a adubação com N, aumentando substancialmente a produção de matéria seca (Van Santen e Sleper, 1996).

A *Festuca pratensis* (Meadow fescue) é uma gramínea extensamente adaptada as terras baixas do centro e do norte da Europa. Esta é usada principalmente para pastejo ou feno (Casler e Santen, 2001). É caracterizada como uma planta de inverno resistente ao frio, sugerindo ser uma planta tolerante ao estresse (Paplauskien et al., 1999). Possui folhas verdes, longas, finas e brilhantes. Não é tão persistente e tão produtiva quanto tall fescue.

O bromus (*Bromus biebersteinii*) é uma gramínea perene de origem Euroasiática comumente empregada como gramínea de pastagem, principalmente nos estados do noroeste dos Estados Unidos e no Canadá (Vogel et al., 1996). A primeira variedade disponível na América do Norte, Regar, foi introduzida da Turkia e registrada nos Estados Unidos em 1966. Depois desta introdução, duas outras variedades, Fleet e Paddock, foram desenvolvidas pelo Agriculture and Agri-Food Canada através da liderança do Dr. Knowles na estação de pesquisa em Saskatoon e lançada em 1987.

É uma gramínea resistente ao inverno e moderadamente tolerante a solos salinos, porém menos tolerante que *Bromus inermis* (smooth bromegrass). Tolera bem a seca, mas morre se for alagada na primavera por 10 dias ou mais. O bromus é recomendado para pastagem de outono, uma vez que cresce bem sob temperaturas frias. Usada de forma complementar com outras gramíneas cultivadas e nativas, ela pode fornecer massa de forragem quando outras gramíneas, como smooth bromegrass são menos produtivas no final do verão e no outono (Knowes et al., 1889).

A gramínea falaris (*Phalaris arundinacea*), no inglês reed canarygrass, é nativa de regiões temperadas do hemisfério norte (Anderson, 1961). Extensas áreas de falaris são frequentemente encontradas no norte dos EUA e no sul do Canadá. Esta é bem adaptada a áreas úmidas de leito sujeitas a inundação (McKenzie , 1951).

A falaris começa o crescimento no início da primavera e se estende até o outono com uma boa distribuição sazonal de rendimento sob fertilidade de N adequada. Esta é uma das gramíneas de estação fria com mais alto rendimento que é usualmente persistente sobre uma vasta série de manejos de colheita e fertilizações de N. Apresenta alto valor nutritivo em estágio de crescimento imaturo e pode ser usada para pastagem, feno ou silagem, contudo esta é provavelmente melhor adaptada para o pastejo. A compatibilidade desta gramínea com leguminosas tem variado muito com o ambiente, com o manejo e com as espécies de leguminosas (Carlson et al., 1996).

2.2. Balanço entre energia e proteína em sistemas de pastagens

Na avaliação da qualidade da forragem a concentração de proteína bruta é uma importante característica. Um alto conteúdo de proteína pode ser conseguido pela escolha de espécies de plantas forrageiras de alta qualidade e procedimentos de manejo. Contudo, a produção de MS e de energia metabolizável são também características primordiais (Myhr et al.,1978).

A digesta que chega no abomaso é completamente diferente do material ingerido pelo ruminante, particularmente no que diz respeito aos carboidratos e proteínas. Uma vez que a fermentação no rúmen pode degradar largamente carboidratos disponíveis, requerendo gliconeogenese, e proteínas na qual a fonte dietética pode ser mais ou menos destruída mas compensada pela síntese de proteína microbiana. Dietas de baixa proteína podem ser suplementadas por síntese microbiana, utilizando ureia endógena reciclada. Enquanto o excesso de proteína em dieta de alto valor proteico é convertida em amônia, que é absorvida e desperdiçada como ureia na urina (Van Soest, 1994).

A sincronia de degradação entre as várias frações de proteína com o fornecimento adequado de carboidratos e suas frações são essenciais para a melhoria da eficiência de uso do nitrogênio pelas vacas. Desequilíbrios entre energia disponível e proteína surgem quando nitrogênio é fornecido em excesso em relação aos requerimentos, proteínas degradáveis no rúmen ou proteínas solúveis são fornecidas em excesso em relação aos carboidratos fermentáveis, dietas são impropriamente equilibradas em relação à proteína não degradável no rúmen (PNDR), ou há quantidades inadequadas ou um desequilíbrio de aminoácidos (Ishler, 2004).

2.2.1. Carboidratos

O termo carboidrato é utilizado para descrever moléculas formadas por hidratos de carbono, possuindo a fórmula geral $C_n(H_2O)_n$. Constituem cerca de 50-80% da matéria seca das forragens e dos cereais. Suas características nutritivas dependem dos componentes dos açúcares, das ligações com lignina polifenólicas e outros fatores físico-químicos (Van Soest, 1994).

Do ponto de vista fisiológico da planta, os carboidratos podem ser classificados dentro de três diferentes categorias: a- açúcares simples e seus compostos envolvidos no metabolismo intermediário da planta, b- compostos de reserva, como sacarose, amido e frutosana, c-polissacarídeos estruturais, principalmente pectina, hemicelulose e celulose (Van Soest, 1994). De uma forma geral os carboidratos podem ser classificados como estruturais e não estruturais.

Os carboidratos não estruturais são localizados dentro das células das plantas e são normalmente mais digestíveis que os carboidratos estruturais (Ishler e Varga, 2001). Estes carboidratos estão relacionados com o metabolismo intermediário, transporte e armazenamento de energia em plantas forrageiras (Smith, 1973). A concentração destes carboidratos é normalmente determinada pela soma das concentrações de sucrose, glicose, frutose, amido e frutosanas (gramíneas) ou pinitol (leguminosas). Outros carboidratos solúveis de menores concentrações são algumas vezes incluídos também (Pelletier et al., 2010).

Os carboidratos solúveis em forragens representam os carboidratos não estruturais ou de reserva da planta. O termo carboidrato solúvel é comumente usado para compostos solúveis em água fria ou em conteúdo gastrointestinal e inclui monossacarídeos, dissacarídeos, oligossacarídeos e alguns polissacarídeos. Os monossacarídeos, glicose e frutose, e os dissacarídeos, sacarose e maltose são os mais importantes carboidratos solúveis das plantas forrageiras (Smith, 1973). De acordo com Woolford (1984) as concentrações de açúcares solúveis da cultura dependem da espécie, das condições climáticas, das aplicações de nitrogênio, da taxa de semeadura e do estádio de desenvolvimento da cultura.

As reservas de carboidratos disponíveis são essenciais para a sobrevivência e para a produção dos tecidos das plantas durante períodos em que a utilização de carboidratos excede a atividade fotossintética. Frutosanas e amido são os mais importantes polissacarídeos de reserva encontrados em plantas (Smith, 1973). A composição de polissacarídeos de armazenamento difere grandemente entre espécies de plantas e, em alguns casos, no que diz respeito a partes de plantas. Ao contrário das gramíneas, o carboidrato preferivelmente estocado em leguminosas é o amido. As frutosanas são estocadas apenas por gramíneas temperadas, na qual o amido ocorre apenas nas sementes e as frutosanas são a forma estocada nas folhas e nas hastes (Van Soest, 1994).

Leguminosass, tais como alfafa, trevo vermelho e trevo branco, são caracterizadas pela acumulação de sacarose e amido. Já gramíneas nativas para latitudes tropicais e subtropicais são caracterizadas pela acumulação de sacarose e amido e gramíneas temperadas, tais como o capim timóteo, festuca e falaris, acumulam sacarose e fructosanas. O capim Timóteo estoca a maior proporção de suas reservas de carboidrato como frutosanas na base das hastes,enquanto a alfalfa estoca a maior parte de suas reservas de carboidratos como amido na raiz (Smith, 1964).

De acordo com o definido por Bailey (1973), os componentes estruturais da planta consistem em sua maior parte de polissacarídeos com quantidades menores de lignina e proteína e diferem dos carboidratos de reserva sendo que uma vez formado não são normalmente remobilizados. Classicamente, os polissacarídeos têm sido agrupados em celulose, hemicelulose e pectinas (Scheller e Ulvskov, 2010).

2.2.2 Proteína

O nitrogênio total da planta é composto por cerca de 60-80% de proteína verdadeira, sendo o restante principalmente completado por nitrogênio não proteico solúvel e uma pequena quantidade de nitrogênio lignificado (Van Soest, 1994). O nitrogênio não proteico (NNP) da forragem consiste de oligopeptídeos, aminoácidos livres, compostos de amônio e outras moléculas pequenas que contribuem rapidamente para o pool de amônia ruminal (Brooderick, 1995).

A maior concentração de proteínas ocorre nas folhas, sendo de alto valor biológico e de composição aminoacídica de elevada qualidade. As proteínas das folhas são relativamente ricas em lisina, mas pobres em metionina e isoleucina, aspecto qualitativo de pouca importância para ruminantes em virtude da intensa degradação proteica e síntese a nível ruminal por força da atividade microbiana (Norton, 1982).

O grau de degradabilidade ruminal da PB pode ser variável entre as diferentes espécies forrageiras, que se refletirá sobre a disponibilidade de compostos nitrogenados em nível ruminal para síntese microbiana, e de aminoácidos no intestino, provenientes da fração proteica dietética não degradada no rúmen (Minson, 1994).

O teor em PB ou N total das leguminosas é superior ao das gramineas (Cruickshank et al., 1992). Contudo, a maior parte da proteína nestas forrageiras é extensivamente degradada no rúmen podendo ser perdida através de amônia quando a energia contida na dieta não é suficiente (Julier et al., 2003).

2.3. Fracionamento de proteínas e carboidratos (CNCPS)

O sistema Cornell de proteínas e carboidratos (CNCPS) foi desenvolvido com o objetivo de avaliar as dietas completas, visando minimizar as perdas dos nutrientes e buscar a maximização da eficiência de crescimento dos microrganismos ruminais (Russel et al., 1992). O sistema (CNCPS) subdivide a proteína e os carboidratos de acordo com sua degradação ruminal e características de digestibilidade.

Os carboidratos são classificados em quatro frações: A, B1, B2 e C. As frações A e B1 correspondem aos carboidratos não estruturais, a fração B2 a porção disponível e a fração C a porção indisponível da parede celular (Sniffen, 1992). A fração A (açúcares e ácidos orgânicos) é rapidamente degradada no rúmen. A fração B1 é composta de amido e pectinas, tendo uma degradação intermediária. A fração B2 corresponde o carboidrato estrutural disponível para degradação ruminal e é obtida subtraindo da fração fibra em detergente neutro a fração C. A fração C corresponde à lignina x 2,4, sendo considerada a porção indisponível da parede celular (Sniffen et al., 1992). A lignina pode representar de 5 a 25% da parede celular, sendo os valores mais elevados encontrados em leguminosas (Van Soest, 1982). A taxa e a extensão da degradação da parede celular da planta varia de acordo com a espécie de forragem e o grau de maturidade (Van Soest, 1994).

O fracionamento de proteína bruta do (CNCPS) fornece uma base para a estimativa da qualidade de proteína de alimentos para gado leiteiro. Este sistema denomina o nitrogênio não proteico como fração A enquanto a proteína verdadeira denominada como fração B é dividida em três frações: B1, B2 e B3 baseadas no decréscimo de solubilidade. A fração B1 é rapidamente degradável no rúmen e constituída de proteínas solúveis; a fração B2 é constituída de proteínas insolúveis em solução tampão e com taxa de degradação intermediária; a fração B3 possui lenta taxa de degradação. O nitrogênio insolúvel em detergente ácido é denotado como fração C, constituída de proteínas insolúveis e não digestíveis no rúmen e nos intestinos (Sniffen et al., 1992).

A suposição pelo uso da solubidade do N em soluções de detergente para fracionamento é a que o N associado ao FDN é a proteína ligada a parede celular, principalmente extensinas covalentemente ligadas a hemicelulose. Enquanto o N insolúvel em detergente ácido é o N associado com lignina e reações de Maillard (Lanzas, 2007). Os teores de nitrogênio ligados aos compostos da parede celular tendem a aumentar com a idade fisiológica da planta, principalmente aquela fração ligada ao FDA (Balsalobre, 2002).

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4. HIPÓTESES E OBJETIVOS GERAIS

A escolha e o aumento da diversidade das espécies de gramíneas e leguminosas utilizadas em consorciação podem proporcionar maior produtividade e maior balanço entre energia e proteína

O objetivo deste trabalho foi avaliar o comportamento de gramíneas e de leguminosas em diferentes sistemas de associação (misturas), por meio de medidas de produção de massa de forragem, da composição nutricional e do balanço entre energia prontamente disponível e proteína.

Segundo as normas do Agronomy Journal

Energy to Protein Ratio of Grass-Legume Binary Mixtures under Frequent Clipping

ABSTRACT

Forages with an improved energy to protein ratio increase nitrogen (N) use efficiency in dairy cows. We studied binary mixtures of one legume and one grass species that can increase the ratio of energy availability to protein degradability under frequent clipping. Timothy (*Phleum pratense* L.), Kentucky bluegrass (*Poa pratensis* L.), tall fescue [*Schedonorus phoenix* (Scop.) Holub], orchardgrass (*Dactylis glomerata* L.), meadow bromegrass (*Bromus biebersteinii* Roemer & J.A. Schultes), and meadow fescue (*Festuca elatior* L.) were seeded with either alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.), or birdsfoot trefoil (*Lotus corniculatus* L.). Carbohydrate and protein fractions (Cornell Net Carbohydrate and Protein System), other nutritive attributes, and dry matter (DM) yield were determined at the first two harvests of the first production year at two sites in eastern Canada. Alfalfa mixtures had greater ratios of water soluble carbohydrates (WSC) to crude protein (CP), and of carbohydrate fractions A+B1 to protein fractions A+B1, than the average of the three legume species. However, they had lower digestibility of neutral detergent fiber (dNDF) and DM (IVTD), greater fiber concentrations, and similar DM yields. Mixtures with meadow fescue and tall fescue had a greater WSC/CP ratio, DM yield, and fiber concentrations than the average of the six grass species. Mixtures of meadow fescue with any legume species, especially with alfalfa, provided the best combination of a high ratio of WSC/CP (0.70) and DM yield and average digestibility. The feasibility of maintaining this desired composition throughout the growing season and over several cropping years remains to be determined.

Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; aNDF, neutral detergent fiber assayed with a heat stable α-amylase; CP, crude protein; CNCPS, Cornell Net Carbohydrate and Protein System; DM, dry matter; dNDF, *in vitro* digestibility of NDF; IVTD, *in vitro* true digestibility of DM; NDF, neutral detergent fiber; NDICP, neutral detergent insoluble crude protein; NIRS, Near Infrared Reflectance Spectroscopy; PCA, principal component analysis; RPD, ratio of standard error of prediction to standard deviation; TN, total nitrogen; TC, total carbohydrates; WSC, water soluble carbohydrates.

Forage species and mixtures should be chosen to suit the soil and climatic characteristics of the farm and the objectives of the farmer (Sanderson et al., 2007), to tolerate several stressors (i.e., defoliation, cold, drought, weed invasion), and to be productive with good nutritive value. Grass-legume mixtures generally provide more consistent forage yield across a wide range of environments throughout the grazing season than grass and legume monocultures (Sleugh et al., 2000; Leep et al., 2002). Forage legumes also fix atmospheric N thereby reducing the need for N fertilization. Their N fixation can also be improved with competition from non-legume species (Nyfeler et al., 2011).

Kentucky bluegrass, meadow fescue, orchardgrass, tall fescue, timothy, and reed canarygrass (*Phalaris arundinacea* L.) are forage grass species that are well adapted to cool seasons and provide high DM yield of good quality forage (Papadopoulos et al., 1995; Kunelius et al., 2003; Oates et al., 2011). Alfalfa, white clover, and birdsfoot trefoil are perennial legume species recommended in eastern Canada but their performance and nutritive value in mixtures with grasses and under grazing are not well documented.

Forage species may affect protein degradability in cattle diets (Cassida et al., 2000). Although forage legumes provide an important source of protein for ruminants, this protein is often poorly utilized by animal. The high concentration of crude protein (CP) in forage legumes, and its fast degradation rate compared with the amount of fermentable organic matter available in the rumen, may lead to inefficient utilization of N by ruminants that results in high N losses to the environment particularly under grazing (Kleen et al., 2011). According to Miller et al. (2001), an increased supply of energy to the rumen would rectify this energy to protein imbalance, favor a higher protein capture, and increase the supply of protein to the ruminant. Enhanced levels of readily-available energy from forages with a high concentration of water soluble carbohydrates (WSC) has been shown to improve the efficiency of N utilization by dairy cows (Cosgrove et al., 2007; Edwards et al., 2007; Brito et al., 2009). For this purpose, binary mixtures with grasses have been frequently used to potentially increase the WSC concentration of forages.

Water-soluble carbohydrates are found mainly inside plant cells. Soon after forage consumption, they become a source of readily-available energy, which allows rumen microbes to process more of the forage protein. The microbial protein can then be used in the production of meat and milk. Parsons et al. (2011) revisited four examples of forage plant breeding and highlighted the importance of the relationship between WSC and CP for efficient N utilization. By plotting data from five different studies, they observed that the WSC:CP ratio was negatively correlated with N excreted in the urine. The Cornell Net Carbohydrate and Protein

System (CNCPS) can be used to synchronise the availability of energy and N to reduce the loss of N compounds and methane production (Sniffen et al., 1992).

Multi-species pastures that include at least one legume species and one grass species may yield more than single-species pastures but research on how this composition affects nutritive value is limited, particularly on the balance between energy and protein. Our objective was to identify binary mixtures, consisting of one legume species and one grass species, that can increase the ratio of energy availability to protein degradability while maintaining yield under frequent clipping.

MATERIALS AND METHODS

The experiment was carried out at two sites: 1) Chapais Research Farm of Agriculture and Agri-Food Canada, Lévis, QC, Canada (46°46' N; 71°12' W, mean elevation: 43 m, soil type: Saint-Aimé fine sandy loam, fine-loamy, mixed, frigid, Typic Humaquept), and 2) Normandin Research Farm of Agriculture and Agri-Food Canada, Normandin, QC, Canada (48º49' N; 72º31' W, mean elevation: 137 m, soil type: Labarre silty clay, fine, mixed, frigid, Humic Crayquept). At the study onset in 2010, soil pH (0–20 cm) and Mehlich-3 (Mehlich, 1984) extractable P and K content (kg ha^{-1}) were, respectively, 5.2, 86, and 199 at Lévis, and 5.9, 143, and 284 at Normandin. Growing degree-days (5ºC basis) and precipitation at both sites are provided in Table 1.

Simple binary mixtures (18) of one grass species and one legume species were compared. The six grass species were: timothy, Kentucky bluegrass, tall fescue, orchardgrass, meadow bromegrass (*Bromus biebersteinii* Roemer & J.A. Schultes), and meadow fescue. They were each seeded with one of three legume species: white clover, birdsfoot trefoil, and alfalfa. Cultivar names and seeding rates of each forage species are provided in Table 2. The treatments were replicated three times in a split-plot layout, with legume species as main plots set out as a Latin square and grass species randomized to the subplots.

The experiment was seeded on 22 and 23 June 2010 at Lévis, and on 2 July 2010 at Normandin. The plot size was 3 × 4 m at Lévis and 3 × 5 m at Normandin. At Lévis, 30 kg N ha^{-1}, 90 kg P ha^{-1}, and 160 kg K ha^{-1} was applied at seeding in 2010, while in 2011 no fertilizer was applied in the spring but 24 kg P ha^{-1} and 116 kg K ha^{-1} was applied after the first harvest. In Normandin, 25 kg N ha^{-1}, 43 kg P ha^{-1}, 83 kg K ha^{-1}, and 1 kg B ha^{-1} was incorporated into the soil before seeding; in 2011, 11 kg P ha^{-1}, 33 kg K ha^{-1}, and 1 kg B ha^{-1} was applied on 3 May but no fertilizer was applied after the first harvest.

At both sites in 2011 were made frequent clipping to a 5-cm height. In each plot, an area of 7.3 m^2 in Lévis and 6.0 m^2 in Normandin was cut using a self-propelled flail forage harvester (Carter MFG Co., Brookston, IN) when timothy reached about 33 cm in height. Because our study focused on the first two months of the growing season, only samples from the first two harvests, representing 43% of the seasonal DM yield (data not shown), were analysed. The first and second harvests from successive regrowths were taken on 2 and 22 June at Lévis, and on 6 and 27 June at Normandin, respectively. Stages of development at cutting (Table 2) were determined according to Simon and Park (1981) for the grass species and Fick and Mueller (1989) for the legume species.

A fresh forage sample of approximately 500 g was taken from each plot, weighed, dried at 55˚C in a force-draft oven to determine dry matter (DM) concentration, and then ground using a Wiley mill (Standard model 3, Arthur H. Thomas Co., Philadelphia, PA) to pass through a 1-mm screen. The frequency grid technique (Vogel and Masters, 2001) was used approximately two weeks after the first harvest to determine the presence of each seeded species in each plot. Two grids of 25 squares (5 × 5 cm each) were placed in each plot. The presence of at least one seeded plant species and one other species was noted for each square. This information represents minimum plant density.

Chemical Analysis

Ground forage samples of the first and second harvests from both sites were scanned by Near Infrared Reflectance Spectroscopy (NIRS) using a NIR system 6500 monochromator (Foss, Silver Spring, MD). A calibration set (n = 60) and a validation set (n = 15) of samples were selected using WinISI III (ver. 1.61) software (Infrasoft International, LLC, Silver Spring, MD) and chemically analysed for concentrations of acid detergent fiber (ADF), neutral detergent fiber assayed with a heat stable α-amylase (aNDF), acid detergent lignin (ADL), total N (TN), CNCPS protein fractions (A, B1, B2, B3, and C), starch, and soluble sugars, as well as for the *in vitro* true digestibility of DM (IVTD) and *in vitro* digestibility of NDF (dNDF).

The ADF and ADL were determined according to Robertson and Van Soest (1981). Neutral detergent fiber (aNDF) was analyzed following Van Soest et al. (1991) with the addition of heat-stable α-amylase. These fiber extractions were determined using the Ankom Filter bag technique. Total N was extracted using a method adapted from Isaac and Johnson (1976). Samples (100 mg) were digested for 60 min at 380ºC in a 1.5-mL mixture of selenious and sulphuric acid (1:42) plus 2 mL of 30% H_2O_2. After cooling, the mixture was diluted to 75 mL with deionized water. Total N was then determined on a QuikChem 8000 Lachat autoanalyser (Zellweger Analytics, Inc., Lachat Instruments, Milwaukee, WI) with method 13-107-06-2-E (Lachat, 2011). Crude protein concentration was estimated as follow: CP = TN × 6.25.

According to CNCPS, N is partitioned into non-protein nitrogen denoted as fraction A, true protein as fraction B, which is further divided into B1, B2, and B3 of rapid, intermediate, and slow rates of ruminal degradation, and unavailable N as the fraction C. The N precipitable with trichloroacetic acid (B1+B2+B3+C), insoluble N in a borate-phosphate buffer (B2+B3+C), neutral detergent insoluble N (B3+C), and acid detergent insoluble N (C) were all chemically determined (Licitra et al., 1996) on the calibration and validation sets of samples.

Soluble sugars in forage samples were extracted in water according to the method described by Suzuki (1971) and Smith (1981). Starch was quantified after its gelatinization and enzymatic hydrolysis by amyloglucosidase, and as glucose equivalent with the p-hydroxybenzoic acid hydrazide method of Blakeney and Mutton (1980). Starch amounts were determined spectrophotometrically by reference to a standard glucose curve.

The IVTD was measured using the method of Goering and Van Soest (1970) based on a 48-h incubation period with buffered rumen fluid, followed by aNDF wash of post-digestion residues. The rumen fluid incubation was performed with ANKON F57 filters bags and an ANKON Daisy II incubator, using the bath incubation procedures outlined by ANKOM Technology Corp. Rumen fluid was obtained from a ruminally fistulated dairy cow that was offered a diet of 37% grass silage, 15% corn silage, 8% hay, 30% corn grain, and 10% concentrate mix formulated to meet the nutritional requirements of a lactating dairy cow expected to produce 10,200 kg milk yr^{-1}. The IVTD (g kg^{-1} DM) and the *in vitro* digestibility of NDF (dNDF; g kg^{-1} aNDF) were calculated as follows:

IVTD = [1 – (post-digestion dry weight following aNDF wash/predigestion dry weight)] × 1000

dNDF = [1 – (post-digestion dry weight following aNDF wash/predigestion dry weight of aNDF)] × 1000

The nutritive attributes, described previously, were thereafter predicted for all forage samples using NIRS (WinISI III software, version 4.0.0.3770, Infrasoft International, LLC, Silver Spring, MD) and the statistics on the performance of these predictions are presented in Table 3. The NIRS predictions were considered successful when the ratio of standard error of prediction to standard deviation (RPD) was greater than 3 (Nie et al., 2009). The RPD was calculated by dividing the standard deviation (SD) of the reference data used in the validation set by the standard error of prediction corrected for bias [SEP(C)]. Using the NIRS predicted values for B1+B2+B3+C, B2+B3+C, B3+C, and C of all forage samples, all protein fractions were then calculated (Licitra et al., 1996). The protein fraction A (PA) was calculated as the difference between the TN and the insoluble N in trichloroacetic acid (B1+B2+B3+C). The protein fraction B1 was calculated as the difference between N precipitable with trichloroacetic acid and insoluble N in a borate-phosphate buffer (B2+B3+C). The protein fraction B2 (PB2) was calculated as the difference between the N insoluble in a borate-phosphate buffer and the N insoluble in neutral detergent (B3+C). The protein fraction B3 (the neutral detergent insoluble N potentially degradable, PB3) was obtained as the difference between the neutral detergent insoluble N and the acid detergent insoluble N (fraction C). All protein fractions were then expressed on a TN basis by multiplying the protein fraction by the TN concentration.

All forage samples were chemically analysed for dry matter and ash concentrations (LECO Corporation, 2009). Crude fat was also determined in all forage samples using the Ankom xt15 Extractor Technology Method (AOCS, 2003).

The CNCPS carbohydrate fractions (A, B1, B2, and C) were calculated according to Sniffen et al. (1992) using the NIRS predicted values of the relevant nutritive attribute. The carbohydrate fraction A (CA) includes soluble sugars and represents the carbohydrate fraction that is degraded rapidly in the rumen. Carbohydrate fraction B1 (CB1) has an intermediate rate of degradation and represents mainly starch and non-starch polysaccharides that are soluble in neutral detergent. Carbohydrate fraction B2 (CB2) is the available cell wall and its degradation rate is slow, and carbohydrate fraction C (CC) represents the unavailable cell wall which is undegradable and indigestible. The concentration of total carbohydrates (TC), expressed in g kg^{-1} DM, was calculated as follows: TC = 1000 – CP – crude fat – ash.

Carbohydrate fractions, expressed as g kg^{-1} of TC, were calculated as follows:

Structural carbohydrates = neutral detergent fiber (aNDF) – neutral detergent insoluble crude protein (NDICP)

Neutral detergent insoluble crude protein = neutral detergent insoluble N × 6.25

Non fiber carbohydrates (NFC) = TC – Structural carbohydrates

Carbohydrate fraction A (CA) = Soluble sugars

Carbohydrate fraction B1 (CB1) = Non Fiber Carbohydrates – soluble sugars

Carbohydrate fraction B2 (CB2) = Structural Carbohydrates – carbohydrate fraction C

Carbohydrate fraction C (CC) = Lignin (ADL) × 2.4

Statistical Analysis

Mean values of DM yield and laboratory analyses were assessed across treatments by analyses of variance (ANOVA) using the GENSTAT 14 statistical software package (VSN International, 2011).Treatments (grass-legume mixtures) were considered fixed effects while sites and replicates were considered random. Because the interest is in a measure of the population response and how it might change relative to the treatment factors, the two harvests were averaged to get the variance homogeneity and provide a more representative value for the mixtures. Differences were considered significant when $P < 0.05$. For each variate, extreme high or low values among forage mixtures were identified after calculating an upper [overall mean + (2.81 × SEM / 2)] and a lower [overall mean – (2.81 × SEM / 2)] limit centered about the overall mean. A principal component analysis (PCA) was used to assess the relationships among variates (DM yield and nutritive attributes) and how variates in these variates are related to forage mixtures. The PCA was performed on the least squares means of the treatments using the correlation matrix method to give equal weight to all variates. The contribution of each variate to a principal component axis can be seen from its loadings (Fig. 1).

RESULTS AND DISCUSSION

Main Effects of Legume and Grass Species

Forage legume species affected all nutritive attributes of the grass-legume mixtures but they did not significantly affect DM yield (Table 4). Alfalfa mixtures had greater starch concentration, and lower concentration of soluble sugars, while white clover and birdsfoot trefoil mixtures had lower starch concentration than the average of the three legume species. Because of their greater concentration of soluble sugars, mixtures with birdsfoot trefoil had greater concentration of carbohydrate fraction A. Mixtures with white clover had lower starch and TC concentrations but greater concentrations of carbohydrate fractions A and B1 (Table 4).

Mixtures with white clover and birdsfoot trefoil had greater TN concentration, while mixtures with alfalfa had lower TN concentration, than the average of the three legume species (Table 4). Julier et al. (2003) in France also report significantly greater CP concentration for white clover and birdsfoot trefoil than for alfalfa. Alfalfa mixtures had greater protein fraction C concentration than the average of the three legume species, and this result was attributed to their greater ADF concentration. Alfalfa mixtures also had greater concentration of protein fraction B1 and lower concentrations of protein fractions A and B2. White clover mixtures had lower concentrations of protein fractions B1, C and B2 and greater concentration of protein fraction B3, and birdsfoot trefoil mixtures had lower concentrations of protein fractions B1, B3 and C and greater concentrations of protein fractions B2 and A, than the average of the three legume species. In their evaluation of protein fractions in alfalfa genotypes, Tremblay et al. (2003) report values of A+B1 (406 g kg^{-1} TN), B2 (520 g kg^{-1} TN), and C (34 g kg^{-1} TN) protein fractions similar to those in our study. The concentration of protein fraction B3, however, was greater in our study with alfalfa-grass mixtures (182 g kg^{-1} TN) than in the study of Tremblay et al. (2003) with alfalfa genotypes (41 g kg^{-1} TN) and cultivars (22 g kg^{-1} TN). Gierus et al. (2012) observed the greatest concentration of protein fraction A in both alfalfa and white clover, and greatest protein fraction C concentration in birdsfoot trefoil single species. This does not agree with the current results since alfalfa mixtures had the lowest protein fraction A and the greatest protein fraction C concentrations as compared with mixtures of white clover or birdsfoot trefoil (Table 4). In the present study, stage of development at harvest combined with the nutritive value of companion grasses may have contributed to greater concentration of protein fraction A in mixtures with birdsfoot trefoil.

Alfalfa mixtures had greater ADF and aNDF concentrations, and lower dNDF and IVTD, while mixtures with white clover and birdsfoot trefoil had lower ADF and aNDF concentrations, and greater IVTD, than the average of the three legume species (Table 4). Alfalfa is known to have a greater aNDF concentration than birdsfoot trefoil and white clover (Cassida et al., 2000; Gierus et al., 2012). The greater IVTD of the white clover mixture may be explained by its low cellulose and lignin concentrations and its greater dNDF. White clover generally has lower concentrations of cellulose, hemicellulose, and lignin than other forage legume species (Whitehead, 1972; Frame and Newbould, 1986). Supporting the results of our study, Sheaffer and Marten (1991) and Sleugh et al. (2000) evaluated Kura clover, which has nutritional attributes similar to white clover (Allison et al., 1985), and report that it had a greater IVTD than alfalfa, birdsfoot trefoil, and their mixtures. Zemenchik et al. (2001) report reduced NDF and ADF concentrations when birdsfoot trefoil was added to grasses.

Because alfalfa is known to have the greatest yield potential of the temperate forage legumes (Frame et al., 1998), mixtures with alfalfa were expected to yield more than those with birdsfoot trefoil and white clover. Alfalfa and alfalfa-grass mixtures yielded more than birdsfoot trefoil and birdsfoot trefoil-grass mixtures in Iowa (Sleugh et al., 2000). In a study of binary mixtures with perennial ryegrass in Germany, mixtures with a grazing-type alfalfa and white clover did not differ significantly in DM yield, but mixtures with alfalfa yielded more than those with birdsfoot trefoil (Gierus et al., 2012). The fact that mixtures with alfalfa did not yield more than those with white clover and birdsfoot trefoil in our study (Table 4) might be explained by the lesser minimum plant density of alfalfa, compared with the average of the three seeded legume species (Table 5).

The grass species in the mixtures affected all nutritive attributes and DM yield (Table 4). Mixtures with meadow fescue, timothy, and tall fescue had great concentrations of soluble sugars, and mixtures with Kentucky bluegrass had greater starch concentrations, than the average of the six grass species. Our results confirm the greater concentration of non-structural carbohydrates of tall fescue previously observed on grass species grown alone (Pelletier et al., 2010). Due to greater concentrations of starch and soluble sugars, mixtures with Kentucky bluegrass and meadow fescue had greater concentrations of carbohydrate fractions B1 and A, respectively.

Mixtures with Kentucky bluegrass and timothy had TN concentrations compared with the average of the six grass species. In agreement with our observations, Durr et al. (2005) and Pelletier et al. (2010) report greater TN concentrations for Kentucky bluegrass than for timothy when both are grown as single species. Kentucky bluegrass and tall fescue mixtures had greater PB2 and PA concentrations, and lower PC and PB3 concentrations, than the average of the three grass species. Kentucky bluegrass mixtures also had the lowest PB1 concentration. Mixtures with orchardgrass had the lowest PB2 and PA concentrations, whereas mixtures with meadow fescue had the lowest PA and PC concentrations.

Mixtures with timothy and Kentucky bluegrass had lower ADF and aNDF concentrations, while mixtures including meadow fescue or tall fescue had greater ADF and aNDF concentrations than the average of the six grass species. Thus, Kentucky bluegrass mixtures had greater IVTD, and tall fescue mixtures had lesser IVTD, than the average of the six grass species. However, mixtures with meadow fescue, orchardgrass, and timothy had greater dNDF. Pelletier et al. (2010) in eastern Canada report greater IVTD and dNDF for timothy than for Kentucky bluegrass in spring growth.

Compared with the average of the six grass species, mixtures with Kentucky bluegrass and timothy had lesser DM yield, while mixtures with meadow fescue and tall fescue had greater DM yield. The greater minimum plant density of meadow fescue, compared with the average of the six seeded grass species (Table 5), may explain its greater DM yield. Meadow fescue in association with white clover also represents one of the most productive mixtures among binary mixtures evaluated in eastern Canada (McKenzie et al., 2005). Our results with tall fescue in mixture with a legume species confirm its greater yield potential in May and June compared with timothy and Kentucky bluegrass when grown alone (Pelletier et al., 2010).

Comparison of the 18 Grass-legume Mixtures

Although the legume × grass interaction was not significant for DM yield, nor for several nutritive attributes (Table 4), we compared the 18 grass-legume mixtures using the PCA because our main objective was to determine the best binary mixture for several attributes of nutritive value and DM yield. The PCA also provides information on the relationships among the nutritive attributes.

The first two principal components explained 73% of the total variation, with 44% and 29% for the first and second components, respectively (Fig. 1). The first component was largely defined by ADF, aNDF, TC, carbohydrate fraction B2, DMY, and protein fraction B1 on the positive side, and by TN, carbohydrate fractions C and B1, PA, and PB2 on the negative side. Attributes within the same group on each side were positively correlated while attributes in opposing groups were negatively correlated. Thus, DM yield was positively correlated with concentrations of ADF, aNDF, souble sugars , and carbohydrate fraction B2 , and negatively correlated with concentrations of TN, protein fractions A and B2, carbohydrate fractions C and B1, and starch and also with IVTD. Positive relationships between DM yield and concentrations of ADF and aNDF, as well as negative relationships between DM yield and TN concentration, have often been reported (Tremblay et al., 2000; Bélanger et al., 2001). These relationships are attributed to changes in the relative proportion of leaves and stems. Mixtures with greater DM yield tend to have greater ADF and aNDF concentrations but lower TN concentration. Some carbohydrate and protein fractions were also involved in the first component. Mixtures with high DM yield had greater concentrations of protein fraction B1 and carbohydrate fraction B2, and lower concentrations of protein fractions A and B2, and carbohydrate fraction B1, than mixtures with low DM yield.

The first component of the PCA mostly defined differences among grass species in the mixtures, separating the mixtures with Kentucky bluegrass on the left (negative), with high TN concentrations and low DM yield, from mixtures with meadow fescue on the right (positive), with greater DM yield and concentrations of NDF and ADF (Fig. 1). Compared with the average values of the 18 mixtures (Table 6), the alfalfa and meadow fescue mixture had a greater DM yield whereas the mixtures of Kentucky bluegrass with either alfalfa or white clover had a lower DM yield. The white clover and Kentucky bluegrass mixture had greater TN concentration and IVTD, and lower ADF and aNDF concentrations, compared with the average of 18 mixtures. In agreement with our observations, Kim and Albrecht (2011) report lower NDF and ADF concentrations, and greater CP concentration, in binary mixtures of

Kura clover (*Trifolium ambiguum)* and bluegrass than of Kura clover and orchardgrass or smooth bromegrass in the first production year.

The second component of the PCA defined a contrast dominated on the positive side by carbohydrate fraction A, dNDF, IVTD, soluble sugars, and protein fraction B3 (Fig. 1). On the negative side, it was largely determined by starch along with protein fraction C and carbohydrate fraction B1. This second component defined differences among legume species, with alfalfa mixtures on the negative side (Table 6, Fig. 1). Alfalfa mixtures had greater starch concentration, and this concentration decreased as the grass species in the mixture went from Kentucky bluegrass, meadow bromegrass, timothy, tall fescue, and orchardgrass to meadow fescue. The birdsfoot trefoil and white clover mixtures were, however, similar and their dNDF and concentration of carbohydrate fraction A increased as grass in the mixtures went from Kentucky bluegrass to meadow fescue (Table 6, Fig. 1).

Although dNDF and concentrations of soluble sugars and protein fraction B3 did not define the first component, they were all closely related to each other on the second PC axis and were associated with mixtures of meadow fescue with white clover and birdsfoot trefoil (Fig. 1). These mixtures provide high digestibility along with greater concentration of readily available sugars and low protein degradability. Their DM yield was also greater than the average of the 18 mixtures (Table 6) and the IVTD of the white-clover meadow-fescue mixture was greater than the average of the 18 mixtures. In general, mixtures of legume species with meadow fescue resulted in forage with high DM yield, digestibility, sugar concentration, and protein degradability.

In this experiment, grass species had a greater influence than the legume species to explain the variation of nutritive attributes and DM yield among the mixtures evaluated. The first principal component explained most of the total variation (44%) and was mainly determined by grass species.

Energy to Protein Ratio

For improved N utilization by dairy cows, a combination of high energy availability for microbial protein synthesis and reduced TN concentration, or reduced N solubility, has been suggested (Bryant et al., 2012). Hence, greater concentrations of A + B1 carbohydrate fractions or WSC along with low concentrations of crude protein or degradable protein (A + B1) should improve N utilization. Enhanced efficiency of N utilization and enhanced milk yield were reported for cows fed alfalfa with greater WSC concentration (Brito et al., 2009) or high sugar grasses (Miller et al., 2001).

A WSC/CP ratio above 0.70 has been proposed to improve dietary N use for milk production and to reduce N loss in urine (Edwards et al., 2007). In the present study, alfalfa mixtures had a greater WSC/CP ratio (Table 7), with lower concentrations of TN and protein fraction A and higher TC concentration (Table 4), than the average of the three legume species. This result also corresponded to a greater (carbohydrates fractions A+B1)/(protein fractions A+B1) ratio (Table 7). The WSC/CP ratio of mixtures with timothy as well as mixtures with orchardgrass, because of their high CA and TN concentrations (Table 4), did not differ significantly from the average of the six grass species (Table 7). However, due to their high CB1 and low PA concentrations, mixtures with timothy had a significantly greater (CA+CB1)/(PA+PB1) ratio than the average of the six grass species. Mixtures with meadow fescue had the greatest WSC/CP ratio. The legume × grass interaction significantly affected the WSC/CP ratio of the mixtures (Table 7), and the alfalfa and meadow fescue mixture had the greatest WSC/CP ratio and a (CA+CB1)/(PA+PB1) ratio greater than the average of the 18 mixtures (Table 8).

Our results from the first two harvests at two sites apply to the first production year in which all seeded species were present following establishment (Table 5). The persistence of some of the seeded species in the northern agricultural areas of eastern Canada, particularly under frequent cutting, might not be sufficient to sustain production over several years. Frequent cutting has been shown to reduce the persistence of alfalfa (Dhont et al., 2004), tall fescue (Drapeau et al., 2007), and meadow fescue (Drapeau and Bélanger, 2009).

CONCLUSIONS

Mixtures of meadow fescue with alfalfa, white clover, or birdsfoot trefoil generally provided forage with the best combination of a high energy to protein ratio and DM yield and average digestibility. The mixture of alfalfa with meadow fescue had the best energy to protein ratio and DM yield but below average digestibility. Mixtures of Kentucky bluegrass with alfalfa or white clover were the least productive, whereas the white clover and Kentucky bluegrass mixture produced the most digestible forage. These results from the first two harvests of the first production year provide useful and novel information on the desired species composition of binary mixtures that combine energy to protein ratio and DM yield. Further research is needed to determine the feasibility of maintaining this desired composition throughout the growing season and over several cropping years.

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FIGURE CAPTION

Figure 1. Diagram of the first two principal component scores calculated for each of the 18 binary legume and grass mixtures (■) and for each nutritive attribute (●) to illustrate the relationship among DM yield and carbohydrate and protein fractions, and among the forage mixtures. ADF = acid detergent fiber; CA = carbohydrate compounds instantaneously degraded in the rumen; CB1 = carbohydrate compounds intermediately degraded; CC = unavailable fiber; CB2 = carbohydrate compounds slowly degraded; DMY = dry matter yield; dNDF = *in vitro* digestibility of NDF; IVTD = *in vitro* true digestibility of DM; aNDF = neutral detergent fiber using α-amylase; PA = non-protein nitrogen compounds instantaneously degraded in the rumen; PB1 = rapidly degraded protein; PB2 = protein with intermediate rate of ruminal degradation; PB3 = slowly degraded protein; PC = bound protein undegraded in the rumen and indigestible in the intestine; SS = soluble sugars; ST = starch; TC = total carbohydrates; TN = total nitrogen. A = alfalfa; B = birdsfoot trefoil; C= white clover; Ti = timothy; Kb = Kentucky bluegrass; Tf = tall fescue; Or = orchardgrass; Mf = meadow fescue; Mb = meadow bromegrass.

Table 1. Average monthly precipitation and accumulated degree-days along with their 30-yr means (1971–2000) at both experimental sites.

		Precipitation (mm)		**Degree-days†**	
Site	**Month**	**2011**	**30-yr mean**	**2011**	**30-yr mean**
Lévis‡	May	130	106	183	199
	June	87	114	354	343
Normandin§	May	96	85	132	145
	June	65	78	303	281

†Degree-days were calculated from a base of 5°C.

‡Reported data were taken at the Quebec airport (46º48` N, 71º23` W, and elevation 74 m) (Environment Canada, 2012).

§Reported data were taken at Normandin (48º51` N, 72º32` W, and elevation 137 m) (Environment Canada, 2012).

Table 2. Cultivars, seeding rates, and stages of development of the legume and grass species for the first two harvests from successive regrowths at Lévis and Normandin.

			Lévis		**Normandin**	
Species	**Cultivar**	**Seeding rate (kg ha$^{-1)}$**	**Harvest 1 (2 June)**	**Harvest 2 (22 June)**	**Harvest 1 (6 June)**	**Harvest 2 (27 June)**
White clover	Milkanova	4	Mid vegetative	Late flower	Mid vegetative	Late flower
Birdsfoot trefoil	AC Langille	8	Mid vegetative	Early bud	Early bud	Late bud
Alfalfa	CRS1001	8	Late vegetative	Late vegetative	Mid vegetative	Late vegetative
Timothy	Express	8	Stem elongation	Sheath elongation	Sheath elongation	Inflorescence emergence
Kentucky bluegrass	Troy	22	Inflorescence emergence	Sheath elongation	Sheath elongation/ booting	Sheath elongation/ inflorescence emergence
Tall fescue	Courtenay	15	Stem elongation	Sheath elongation	Sheath elongation	Sheath elongation/ inflorescence emergence
Orchardgrass	Killarney	12	Booting/inflorescence emergence	Sheath elongation	Sheath elongation	Inflorescence emergence
Meadow fescue	Pardel	15	Stem elongation/ booting	Sheath elongation	Sheath elongation	Inflorescence emergence
Meadow bromegrass	Fleet	15	Inflorescence emergence	Sheath elongation	Sheath elongation	Inflorescence emergence

Table 3. Statistics on the performance of near infrared spectroscopy (NIRS) to predict the nutritive attributes of the validation set of forage samples.

Attribute†	**n‡**	**Slope**	**Mean**	**SD**	**SEP**	**RSQ**	**SEP(C)**	**RPD**
ADF (g kg^{-1} DM)	15	0.94	291	35	10	0.94	9.1	3.9
aNDF (g kg^{-1} DM)	15	1.01	416	66	15	0.96	13	5.1
ADL (g kg^{-1} DM)	14	0.99	30	8.1	2.0	0.94	2.0	4.1
TN (g kg^{-1} DM)	15	1.00	31	5.1	0.79	0.98	0. 8	6.5
Protein fractions								
B1+B2+B3+C (g TN kg^{-1} DM)	15	1.02	24	3.2	0.84	0.94	0.8	4.0
B2+B3+C (g TN kg^{-1} DM)	15	1.00	21	4.2	0.80	0.96	0.8	5.1
B3+C (g TN kg^{-1} DM)	15	1.10	6.8	1.97	0.46	0.95	0.47	4.2
C (g TN kg^{-1} DM)	14	0.93	1.2	0.36	0.15	0.81	0.16	2.3
Starch (g kg^{-1} DM)	15	0.95	34	32	8.91	0.93	8.7	3.7
Soluble sugars (g kg^{-1} DM)	15	0.91	103	30	10	0.92	9.0	3.3
IVTD (g kg^{-1} DM)	15	0.02	910	21	7.4	0.90	6.6	3.2
dNDF (g kg^{-1} aNDF)	15	0.99	797	26	7.7	0.93	7.1	3.7

†ADF = acid detergent fiber; aNDF = neutral detergent fiber using α-amylase; ADL = acid detergent lignin; TN = total N; B1 = rapidly degraded protein; B2 = protein with intermediate rate of ruminal degradation; B3 = slowly degraded protein; C = bound protein undegraded in the rumen and indigestible in the intestine; B1+B2+B3+C = true protein precipitated with tungstic acid; B2+B3+C = N insoluble in a borate-phosphate buffer; B3+C = N insoluble in neutral detergent; IVTD = *in vitro* true digestibility of dry matter, and; dNDF = *in vitro* digestibility of NDF.

‡n = number of samples in the validation set; SD = standard deviation; SEP = standard error of prediction; SEP(C) = standard error of prediction corrected for the bias; RSQ = coefficient of determination for the prediction; RPD = ratio of prediction to deviation (SD/SEP(C)).

Table 4. Means of all nutritive attributes and DM yield for the main effects of three legume species and six grass species. Nutritive attributes and DM yield were sorted according to the first principal component scores†.Values are the average of the first two harvests at two sites.

	ADF‡	aNDF	TC	CB2	PB1	DMY	PC	SS	dNDF	PB3	ST	CA	PB2	IVTD	PA	CC	CB1	TN
Main effects		—— g kg^{-1} DM ——		g kg^{-1} TC	g kg^{-1} TN	t ha^{-1}	g kg^{-1} TN	g kg^{-1} DM	g kg^{-1} aNDF	g kg^{-1} TN	g kg^{-1} DM	g kg^{-1} TC	g kg^{-1} TN	g kg^{-1} DM	g kg^{-1} TN	– g kg^{-1} TC –		g kg^{-1}DM
Legumes																		
White clover	269	410	655	447	116	1.38	34	107	**820**	**212**	25	**163**	398	**932**	241	91	**299**	**37**
Birdsfoot trefoil	271	422	665	446	105	1.42	35	**112**	785	180	34	**168**	**437**	**920**	**255**	**111**	276	**35**
Alfalfa	**294**	**445**	**709**	**467**	**144**	1.41	**45**	102	775	182	**52**	145	404	904	226	98	291	30
SEM (n=36, df=81)	2.38	3.97	2.99	5.10	3.66	0.031	0.58	2.09	2.11	1.43	1.64	2.78	2.43	0.94	2.20	0.99	4.90	0.31
upper limit	281	431	681	461	127	1.45	39	110	796	193	39	162	416	920	243	101	296	34
lower limit	275	420	672	446	117	1.36	37	104	790	189	34	154	409	917	237	98	282	33
Grasses																		
Timothy	266	411	675	419	120	1.28	**41**	**113**	**804**	**196**	38	**168**	415	920	231	**112**	**301**	**34**
Kentucky bluegrass	245	365	643	372	108	1.06	35	95	762	177	**51**	149	**429**	**922**	**254**	**114**	**365**	**38**
Tall fescue	**296**	**450**	**693**	**498**	120	**1.52**	34	**112**	790	177	28	163	**420**	914	**255**	86	255	32
Orchardgrass	**291**	**453**	**691**	**480**	**135**	1.42	**44**	105	**805**	**214**	34	152	381	919	227	100	268	32
Meadow fescue	**296**	**457**	**696**	**504**	128	**1.70**	36	**122**	**809**	192	31	**174**	415	920	233	82	239	31
Meadow bromegrass	276	419	661	448	119	1.44	38	95	790	192	39	145	**418**	918	243	**104**	**303**	36
SEM (n=18, df=81)	3.37	5.61	4.23	7.21	5.18	0.044	0.82	2.96	2.98	2.03	2.32	3.93	3.43	1.33	3.11	1.40	6.93	0.43
upper limit	283	434	682	464	129	1.47	39	111	797	194	40	164	418	921	245	102	298	34
lower limit	273	418	670	443	114	1.34	37	103	789	189	33	153	408	917	236	98	279	33
Overall mean	278	426	676	453	122	1.41	38	107	793	191	37	158	413	919	240	100	289	34

Source of variation	**df**	**F probability**																
Legume	2	***	***	***	**	***	ns	***	**	***	***	***	***	***	***	***	***	**	***
Grass	5	***	***	***	***	*	***	***	***	***	***	***	***	***	**	***	***	***	***
Legume × grass	10	ns	ns	Ns	ns	ns	ns	ns	ns	*	**	*	ns	ns	*	**	***	ns	*

†For each attribute, the extreme high and low values among forage mixtures are noted in bold or underlined, respectively, after calculating an upper [overall mean + (2.81 × SEM / 2)] and a lower [overall mean – (2.81 × SEM / 2)] limit, centered about the overall mean.

‡ADF = acid detergent fiber;a NDF = neutral detergent fiber using α-amylase; TC = total carbohydrates; CB2 = carbohydrates compounds slowly degraded; DMY = dry matter yield; PB1 = rapidly degraded protein; PC = bound protein undegraded in the rumen and indigestible in the intestine; SS = soluble sugars; dNDF = *in vitro* digestibility of NDF; PB3 = slowly degraded protein; CA = carbohydrate compounds instantaneously degraded in the rumen; ST = starch; IVTD = *in vitro* true digestibility; PB2 = protein with intermediate rate of ruminal degradation; PA = non protein N compounds instantaneously degraded in the rumen; CB1 = carbohydrate compounds intermediately degraded; CC = unavailable fiber; TN = total N.

ns = not significant, * = Significant at the 0.05 probability level, ** = Significant at the 0.01 probability level, *** = Significant at the 0.001 probability level.

Table 5. Minimum plant density† of each species after the first harvest of the first production year. Values are the average at two sites‡.

Main effects	Seeded legume	Seeded grass	Other species
	plant m^{-2}		
Legumes			
White clover	**39.5**	37.7	11.4
Birdsfoot trefoil	**38.6**	38.5	**19.4**
Alfalfa	34.5	37.9	**21.2**
SEM§ (n = 36, df = 81)	0.45	0.41	0.78
upper limit	38.1	38.6	18.4
lower limit	36.9	37.4	16.2
Grasses			
Timothy	38.0	37.2	17.6
Kentucky bluegrass	38.0	37.8	**22.1**
Tall fescue	37.7	**39.4**	15.9
Orchardgrass	36.4	**39.2**	14.6
Meadow fescue	37.0	**39.5**	15.4
Meadow bromegrass	37.9	35.1	18.6
SEM (n=18, df=81)	0.64	0.58	1.10
upper limit	38.4	38.8	18.8
lower limit	36.6	37.2	15.8
Overall mean	37.5	38.0	17.3

Source of variation	**df**		**F probability**	
Legume	2	***	ns	***
Grass	5	ns	***	***
Legume × grass	10	ns	ns	ns

†Determined as described by Vogel and Masters (2001).

‡For each attribute, the extreme high and low values within legume and grass mixtures are noted in bold or underlined, respectively, after calculating an upper [overall mean + (2.81 × SEM / 2)] and a lower [overall mean – (2.81 × SEM / 2)] limit, centered on the overall mean.

§Standard error of the mean.

¶Degrees of freedom.

***Significant at the 0.001 probability level.

Table 6. The two principal component (PC) scores and means of all nutritive attributes and DM yield of 18 simple legume-grass mixtures sampled at the first two harvests and at two sites. The legume-grass mixtures and nutritive attributes and DM yield were sorted according to the first principal component scores†. Values are the average of the first two harvests at two sites.

Simple mixtures	PC 1	PC 2	ADF‡	aNDF	TC	CB2	PB1	DMY	PC	SS	dNDF	PB3	ST	CA	PB2	IVTD	PA	CC	CB1	TN
	(λ_1 =44%)	(λ_2 = 29%)	—— g kg^{-1} DM ——			g kg^{-1} TC	g kg^{-1} TN	Mg ha^{-1}	g kg^{-1} TN	g kg^{-1} DM	g kg^{-1} aNDF	g kg^{-1} TN	g kg^{-1} DM	g kg^{-1} TC	g kg^{-1} N	g kg^{-1} DM	g kg^{-1} N	— g kg^{-1} TC —		g kg^{-1} DM
AOr	**4.20**	-2.20	**308**	**477**	**722**	**499**	**158**	1.41	**51**	104	786	**208**	**47**	144	367	905	215	98	260	28
AMf	**4.00**	0.36	**308**	**474**	**721**	**517**	**139**	**1.74**	**41**	**118**	796	183	39	164	412	909	225	83	236	28
ATf	**3.02**	-0.55	**311**	**471**	**719**	**515**	133	1.48	39	109	778	166	35	153	414	902	**249**	82	251	29
BMf	**2.14**	**2.57**	**294**	**461**	**687**	**509**	118	**1.67**	35	**130**	**805**	187	31	**186**	**429**	919	243	86	219	31
ATi	**2.03**	-2.51	284	**439**	**721**	436	**140**	1.28	**50**	**115**	784	192	**57**	160	407	902	211	**109**	296	29
CMf	1.37	**2.72**	**286**	435	679	**487**	128	**1.69**	33	**117**	**827**	**207**	24	**172**	403	**931**	229	79	262	34
AMb	0.91	-3.75	**289**	427	685	449	**150**	1.47	**44**	83	769	181	**56**	121	408	905	218	**105**	**325**	32
BOr	0.86	0.35	284	**447**	681	469	119	1.39	**42**	110	800	**203**	30	161	407	920	229	**109**	261	32
COr	0.65	1.40	280	435	669	**471**	126	1.47	39	102	**829**	**230**	24	152	369	**931**	236	94	283	**35**
BTf	0.63	**1.67**	**290**	**448**	**690**	**490**	108	**1.51**	32	**122**	782	166	27	**178**	**446**	916	**259**	95	240	32
CTf	0.38	**1.89**	**287**	432	669	**488**	120	**1.57**	32	104	**811**	**201**	21	156	401	**925**	**257**	80	275	**35**
CMb	-1.35	**2.17**	269	414	648	453	101	1.35	34	107	**823**	**215**	25	165	406	**932**	244	90	293	**38**
AKb	-1.65	-5.56	266	383	685	385	**141**	1.07	**42**	86	737	164	**77**	125	413	902	239	**111**	**380**	33
BMb	-1.93	-0.33	269	416	648	442	106	**1.51**	36	96	777	180	35	148	**439**	918	**267**	**117**	293	**37**
CTi	-2.00	**1.51**	256	388	645	409	122	1.26	36	109	**828**	**213**	23	**170**	400	**934**	229	102	**319**	**38**
BTi	-2.27	0.67	257	407	661	411	98	1.30	37	**115**	**801**	183	34	**175**	**438**	**922**	**254**	**126**	289	**36**
CKb	-4.85	0.69	236	356	620	376	101	0.96	32	100	**802**	**208**	31	162	411	**938**	**248**	99	**364**	**42**
BKb	-6.15	-1.09	232	355	625	356	82	1.15	31	100	745	160	**44**	160	**462**	**927**	**275**	**132**	**353**	**40**
Overall mean	0.00	0.00	278	426	676	453	122	1.41	38	107	793	191	37	158	413	919	240	100	289	34
SEM (n=6,df=81)¶	1.00	1.00	5.8	9.7	7.3	12.5	9.0	0.076	1.4	5.1	5.2	3.5	4.0	6.8	6.0	2.3	5.4	2.4	12.0	0.8
upper limit	1.41	1.41	286	439	687	471	134	1.51	40	114	801	196	42	168	421	922	248	103	306	35
lower limit	-1.41	-1.41	270	412	666	436	109	1.30	36	100	786	186	31	149	405	916	233	96	272	33

†For each attribute, the extreme high and low values among forage mixtures are noted in bold or underlined, respectively, after calculating an upper [overall mean + (2.81 × SEM / 2)] and a lower [overall mean – (2.81 × SEM / 2)] limit, centered about the overall mean.

‡ADF = acid detergent fiber; aNDF = neutral detergent fiber using α-amylase ; TC = total carbohydrates; CB2 = carbohydrates compounds slowly degraded; DMY = dry matter yield; PB1 = rapidly degraded protein; PC = bound protein undegraded in the rumen and indigestible in the intestine; SS = soluble sugars; dNDF = *in vitro* digestibility of NDF; PB3 = slowly degraded protein; CA = carbohydrate compounds instantaneously degraded in the rumen; ST = starch; IVTD = *in vitro* true digestibility; PB2 = protein with intermediate rate of ruminal degradation; PA = non-protein nitrogen compounds instantaneously degraded in the rumen; CB1 = carbohydrate compounds intermediately degraded; CC = unavailable fiber; TN = total nitrogen.

§C = white clover, B = birdsfoot trefoil, A = alfalfa, Ti = timothy, Kb = Kentucky bluegrass, Tf = tall fescue, Or = orchardgrass, Mf = meadow fescue, Mb = meadow bromegrass.

¶Standard error of the mean.

Table 7. Ratios of WSC/CP and (CA+CB1)/(PA+PB1) †for the main effects of three legume and six grass species. Values are the average of the first two harvests at two sites‡.

Main effects	g WSC g^{-1} CP	g (CA+CB1) g^{-1} (PA+PB1)
Legumes		
White clover	0.47	3.75
Birdsfoot Trefoil	0.52	3.99
Alfalfa	**0.57**	**4.60**
SEM (n=36, df=81)§	0.013	0.064
upper limit	0.54	4.20
lower limit	0.50	4.02
Grasses		
Timothy	0.54	**4.39**
Kentucky bluegrass	0.40	3.93
Tall fescue	**0.57**	4.05
Orchardgrass	0.54	4.11
Meadow fescue	**0.63**	4.23
Meadow bromegrass	0.44	3.95
SEM (n=18, df=81)	0.018	0.090
upper limit	0.55	4.24
lower limit	0.50	3.98
Overall mean	0.52	4.11

Source of variation	df		F probability
Legume	2	***	***
Grass	5	***	**
Legume × grass	10	**	ns

† CA = carbohydrate compounds instantaneously degraded in the rumen; CB1 = carbohydrate compounds intermediately degraded; CP = crude protein; PA = non-protein nitrogen compounds instantaneously degraded in the rumen; PB1 = rapidly degraded protein; WSC, water soluble carbohydrates.

‡For each attribute within legume and grass species, the extreme high and low values are noted in bold or underlined, respectively, after calculating an upper [overall mean + (2.81 × SEM / 2)] and a lower [overall mean – (2.81 × SEM / 2)] limit, centered about the overall mean.

§Standard error of the mean.

ns = not significant, ** = Significant at the 0.01 probability level, *** = Significant at the 0.001 probability level.

Table 8. Ratios of WSC/CP and (CA+CB1)/(PA+PB1)† for the 18 simple legume-grass mixtures. Values are the average of two harvests at two sites‡.

Simple mixtures	**g WSC g^{-1} CP**	**g (CA+CB1) g^{-1} (PA+PB1)**
Alfalfa/Orchardgrass	**0.60**	**4.56**
Alfalfa/Meadow fescue	**0.70**	**4.67**
Alfalfa/Tall fescue	**0.62**	**4.41**
Birdsfoot trefoil/Meadow fescue	**0.63**	4.05
Alfalfa/Timothy	**0.65**	**5.28**
White clover/Meadow fescue	0.57	3.98
Alfalfa/Meadow bromegrass	0.41	4.15
Birdsfoot trefoil/Orchardgrass	0.53	4.01
White clover/Orchardgrass	0.48	3.76
Birdsfoot trefoil/Tall fescue	**0.62**	4.12
White clover/Tall fescue	0.48	3.62
White clover/Meadow bromegrass	0.47	3.72
Alfalfa/Kentucky bluegrass	0.42	**4.52**
Birdsfoot trefoil/Meadow bromegrass	0.43	3.98
White clover/Timothy	0.46	3.79
Birdsfoot trefoil/Timothy	0.51	4.12
White clover/Kentucky bluegrass	0.39	3.63
Birdsfoot trefoil/Kentucky bluegrass	0.40	3.65
Overall mean	0.52	4.11
SEM (n=6, df=81)¶	0.031	0.156
upper limit	0.56	4.33
lower limit	0.48	3.89

† CA = carbohydrate compounds instantaneously degraded in the rumen; CB1 = carbohydrate compounds intermediately degraded; CP = crude protein; PA = non-protein nitrogen compounds instantaneously degraded in the rumen; PB1 = rapidly degraded protein; WSC, water soluble carbohydrates.

‡For each attribute among forage mixture, the extreme high and low values are noted in bold or underlined, respectively, after calculating an upper [overall mean + (2.81 × SEM / 2)] and a lower [overall mean – (2.81 × SEM / 2)] limit, centered about the overall mean.

¶Standard error of the mean.

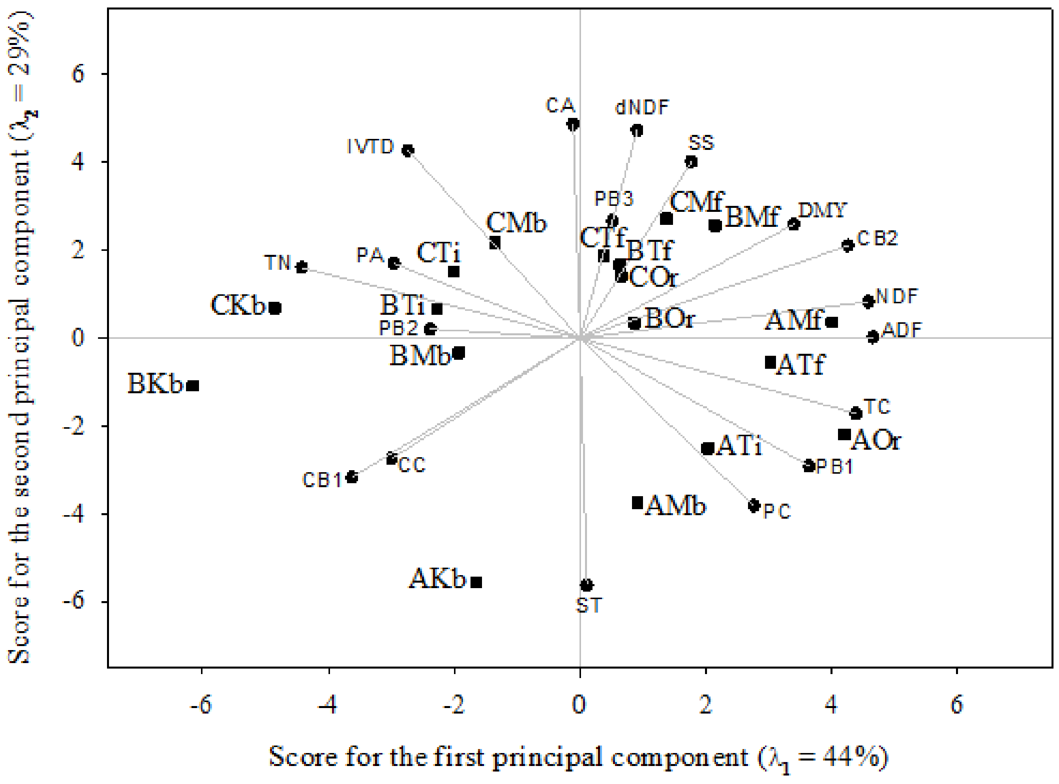
Score for the second principal component ($\lambda_2 = 29\%$)
6
4
2
0
-2
-4
-6
-6
-4
-2
0
2
4
6
Score for the first principal component ($\lambda_1 = 44\%$)
CA
dNDF
IVTD
SS
PB3
CMf
BMf
DMY
CMb
CTf
BTf
CB2
TN
PA
CTi
COr
CKb
BTi
NDF
PB2
BOr
AMf
ADF
BMb
ATf
BKb
TC
AOr
ATi
CC
PB1
CB1
AMb
PC
AKb
ST

Fig. 1

Segundo as normas do Agronomy Journal

Nutritional value of complex forage mixtures made of one legume and three or four grass species and grown for pasture in the Northern latitudes

ABSTRACT

The use of legume grass complex mixtures may increase the yield of pastures but little is known on their nutritive value, particularly the balance between readily-available energy and protein. Our objective was to determine the complex mixture that provides the best balance between energy and protein while having DM yield. Four grass-species mixes were seeded with either a grazing type alfalfa (A, *Medicago sativa* L. cv. CRS1001) or birdsfoot trefoil (B, *Lotus corniculatus* L. cv. AC Langille). The grass-species mixes were TiMfRcKb, KbTfOrMb, MfTiKb, and RcKbTfMb where Ti stands for timothy (*Phleum pratense* L. cv. Express), Mf for meadow fescue (*Festuca elatior* L. cv. Pardel), Rc for reed canarygrass (*Phalaris arundinacea* L. cv.), Kb for Kentucky bluegrass (*Poa pratensis* L. cv. Troy), Tf for tall fescue [*Schedonorus phoenix* (Scop.) Holub], Or for orchardgrass (*Dactylis glomerata* L.cv. Killarney), and Mb for meadow bromegrass (*Bromus biebersteinii* Roemer & J.A. Schultes, cv. Fleet). Alfalfa-based mixtures had greater ADF, aNDF, and TC concentrations, greater WSC/CP and (carbohydrate fractions A+B1)/(protein fractions A+B1) ratios, and lower concentrations of TN and protein fraction A than the average of the eight complex mixtures. The grass species mixes with meadow fescue had a greater DM yield than the average of the eight complex mixtures. The MfTiKb grass species mix had a lower ADF concentration and greater dNDF and IVTD, whereas the TiMfRcKb grass species mix had greater WSC/CP and carbohydrate fractions B1+A/ protein fractions B1+A ratios than the average of the eight complex mixtures. The ATiMfRcKb complex mixture had the greatest WSC/CP ratio. The complex mixtures with alfalfa and meadow fescue provided the best combination of a high WSC/CP ratio and DM yield. The feasibility of maintaining this desired composition throughout the growing season and over several cropping years remains to be determined.

Abbreviations: A, grazing type alfalfa; ADF, acid detergent fiber, ADL, acid detergent lignin; aNDF, neutral detergent fiber assayed with a heat stable α-amylase; Bt, birdsfoot trefoil; CP, crude protein; CNCPS, Cornell Net Carbohydrate and Protein System; dNDF, *in vitro* digestibility of NDF; IVTD, *in vitro* true digestibility of DM; Kb, Kentucky bluegrass; Mf, meadow fescue; Mb, meadow bromegrass; NDF, neutral detergent fiber; NDICP, neutral detergent insoluble crude protein; NIRS, Near Infrared Reflectance Spectroscopy; O, orchardgrass; PCA, principal component analysis; Rc, reed canarygrass; Tf, tall fescue; Ti, timothy; TN, total nitrogen; TC, total carbohydrates; WSC, water soluble carbohydrates.

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Ecological and agronomic studies suggest that increase crop diversity in species-poor intensive systems might improve their provision of ecosystem services (Kirwan et al., 2007). Mixed pastures can substantially affect herbage distribution and weed invasion (Sanderson et al., 2005). The interaction among particular species plays a significant role in achieving greater forage productivity (Skinner et al., 2006). Tracy and Sanderson (2004) reported averaged forage yields lower than 4 Mg ha^{-1} yr^{-1} in mixtures having one or two species while mixtures with three species or more averaged more than 10 Mg ha^{-1} yr^{-1}.

The positive relationship between plant diversity and production in grassland may involve complementarity resource use among plants where different species complement each other by having different rooting depths, leaf architecture, and growth rates (Tracy and Sanderson, 2004). The choice of forage species may influence protein degradability in cattle diets (Cassida et al., 2000). However, the selection and combination of forage species for improved DM yield is a challenge in designing diverse pasture systems (Sanderson et al., 2007).

Legumes have contributed to improve forage quality and productivity of mixtures, especially because of the potential for self-sufficiency for N, by symbiotically fixing atmospheric N. However, rapid and extensive ruminal degradation of proteins in legume and grass forages generally leads to decreased protein efficiency (Broderick, 1995). Once in an environment where energy is limiting but there is an excess of peptides and amino acids of plant origin, the rumen microbial population uses amino acids for energy and liberates ammonia through deamination (Kingston-Smith and Theodorou, 2000).

The rumen fermentation and post rumen digestion depend on total concentrations and degradation rates of carbohydrates and proteins (Mello and Nornberg, 2004). With a greater supply of readily fermentable carbohydrates in the rumen, more non-protein N and amino acids can be taken up by the microorganisms and incorporated into microbial proteins. It is proposed that forages with high WSC may improve the balance and synchrony of the carbon (C) and nitrogen (N) (Miller et al., 2001) and increase microbial protein production in the rumen and animal productivity (Parsons et al., 2011). Berthiaume et al. (2010) observed that increasing nonstructural carbohydrates (NSC) in alfalfa significantly decreased ruminal pH and NH_3-N concentration. By increasing the NSC proportion and reducing the NDF concentration of the diets, Stokes et al. (1991) obtained greater microbial protein yield and proportion of CHO digested. By using the CNCPS, it is possible to synchronise between the availability of energy and N in an attempt to reduce the loss of N compounds and methane production, which allows estimating ruminal escape of nutrients (Sniffen et al., 1992).

The use of the legume grass complex mixtures may increase the yield of pastures but little is known on their nutritive value, particularly on the balance between readily-available energy and protein. Our objective was to determine what complex mixture made of three or four grass species and one legume can provide the best balance between readily-available energy and protein while having good yield.

MATERIALS AND METHODS

The experiment was carried out at two sites: 1) Chapais Research Farm of Agriculture and Agri-Food Canada: Levis, QC, Canada (46° 46'21''N; 71 12'18''W, mean elevation: 43 m, soil type: Saint-Aimé fine sandy loam) and 2) Normandin Research Farm of Agriculture and Agri-Food Canada: Normandin, QC, Canada (48º 49'60" N, 72º 31'60" W, mean elevation: 137 m, soil type: Labarre silty clay). At the beginning of the study in 2010, soil pH (0-20 cm) and Mehlich-3 (Mehlich, 1984) extractable P and K content (kg ha^{-1}) were, respectively, 5.1, 63, and 291 at Lévis, and 5.9, 143, and 284 at Normandin. Growing degree-days (5º basis) and precipitation at both sites are presented in Table 1. In this experiment, eight complex mixtures made of one legume and three or four grass species were compared. Four grass-species mixes were seeded with either a grazing type alfalfa or birdsfoot trefoil. The grass-species mixes were TiMfRcKb, KbTfOrMb, MfTiKb, and RcKbTfMb where Ti stands for timothy, Mf for meadow fescue, Rc for reed canarygrass, Kb for Kentucky bluegrass, Tf for tall fescue, Or for orchardgrass, and Mb for meadow bromegrass.

The experiments were seeded on 22 and 23 June 2010 at Lévis and on 02 July 2010 at Normandin. Alfalfa and birdsfoot trefoil were both seeded at the rate of 6 kg ha^{-1}. The TiMfRcKb grass species mix was seeded at the rate of 4, 7, 2, and 3 kg ha^{-1} of Ti, Mf, Rc, and Kb, respectively. The KbTfOrMb mix was seeded at the rate of 3, 6, 3, and 7 kg ha^{-1} of Kb, Tf, Or, and Mb, respectively. The MfTiKb mix was seeded at the rate of 7, 5, and 4 kg ha^{-1} of Mf, Ti, and Kb, respectively. And finally, the RcKbTfMb mix was seeded at the rate of 2, 3, 6, and 5 kg ha^{-1} of Rc, Kb, Tf, and Mb, respectively. The plot size was 3 × 4 m at Lévis and 3 × 5 m at Normandin. At Lévis, 30 kg N ha^{-1} as calcium ammonium nitrate, 90 kg P ha^{-1} as triple superphosphate, and 160 kg K ha^{-1} as muriate of potassium were applied at seeding in 2010. No fertilizers were applied in the spring of 2011. After the first cut, 35 kg P ha^{-1} and 83 kg K ha^{-1} were applied. In Normandin, 25 kg N ha^{-1}, 43 kg P ha^{-1}, 83 kg K ha^{-1}, and 1 kg B ha^{-1} were incorporated into the soil before seeding. In spring 2011, 11 kg P ha^{-1}, 33 kg K ha^{-1}, and 1 kg B ha^{-1} were applied on 3 May.

At both sites in 2011 were made frequent clipping to a 5-cm height. In each plot, an area of 7.3 m^2 in Lévis and 6.0 m^2 in Normandin was harvested with a self-propelled flail forage harvester (Carter MFG Co., Brookston, IN) when timothy reached around 33 cm in height., Because our study focused on the first two months of the growing season, only samples from the first two harvests were analysed. The first and second harvests from sucessive regrowths were taken on 2 and 22 June at Lévis, and on 6 and 27 June at Normandin, respectively. Stages of development (Table 2) at harvests of the grasses were determined according to Simon and Park (1991) whereas those of legumes were determined based on Fick and Mueller (1989).

A fresh sample of approximately 500 g was taken from each plot at each harvest, weighed, dried at 55˚C in a force-draft oven to determine the DM concentration, and then ground using a Wiley mill (Standard model 3, Arthur H. Thomas Co., Philadelphia, PA) to pass through a 1-mm screen. The frequency grid technique (Vogel and Masters, 2001) was used approximately two weeks after the first harvest to assess the presence of each seeded species in each plot. Two grids of 25 squares of 5 × 5 cm each were placed in each plot. The presence of at least one plant of the seeded species and other species was noted for each square. The information generated by this technique represents the minimum plant density of each seeded species.

Chemical Analyses

Ground forage samples of the first and the second harvests from both sites were scanned by Near Infrared Reflectance Spectroscopy (NIRS) using a NIRsystem 6500 monochromator (Foss, Silver spring, MD). A calibration set (n = 60) and a validation set (n = 15) of samples were selected using the WinISI III (ver. 1.61) software (Infrasoft International, LLC, Silver Spring, MD) and chemically analysed for concentrations of acid detergent fibre (ADF), neutral detergent fibre assayed with a heat stable α-amylase (aNDF), acid detergent lignin (ADL), total N (TN), CNCPS protein fractions (A, B1, B2, B3, and C), starch, and soluble sugars, as well as for the *in vitro* true digestibility of DM (IVTD) and *in vitro* digestibility of NDF (dNDF).

The ADF and ADL was determined according to Robertson and Van Soest (1981). Neutral detergent fiber (aNDF) was analyzed following Van Soest et al. (1991) with addition of heat-stable α-amylase. These fiber extractions were determined using the Ankom filter bag technique. Total N was extracted using a method adapted from Isaac and Johnson (1976). Samples (100 mg) were digested for 60 min at 380ºC in a 1.5-mL mixture of selenious and sulphuric acid (1:42) plus 2 mL of 30% H_2O_2. After cooling, the mixture was diluted to 75 mL with deionized water. Total N was then determined on a QuikChem 8000 Lachat autoanalyser (Zellweger Analytics, Inc., Lachat Instruments, Milwaukee, WI) using the method 13-107-06-2-E (Lachat, 2011). Crude protein (CP) concentration was estimated as follow: CP = TN × 6.25.

According to CNCPS, N is partitioned into non-protein nitrogen (NPN) denoted as fraction A, true protein as fraction B, which is further divided into B1, B2, and B3 of rapid, intermediate, and slow rates of ruminal degradation, and unavailable N as the fraction C. The N precipitable with trichloroacetic acid (B1+B2+B3+C), insoluble N in a borate-phosphate buffer (B2+B3+C), neutral detergent insoluble N (B3+C), and acid detergent insoluble N (C) were all chemicall determined (Licitra et al., 1996) on the calibration and validation sets of samples.

Soluble sugars in forage samples were extracted in water according to the method described by Suzuki (1971) and Smith (1981). Starch was quantified after its gelatinization and enzymatic hydrolysis by amyloglucosidase, and as glucose equivalent with the p-hydroxybenzoic acid hydrazide method of Blakeney and Mutton (1980). Starch amounts were determined spectrophotometrically by reference to a standard glucose curve.

The IVTD was measured using the method of Goering and Van Soest (1970) based on a 48-h incubation with buffered rumen fluid followed by an aNDF determination of the post digestion residues. The rumen fluid incubation was performed with Ankom F57 filter bags and an Ankom Daisy II incubator, using the bath incubation procedures outlined by Ankom Technology Corp. Rumen fluid was obtained from a ruminally fistulated dairy cow that was offered a diet of 37% grass silage, 15% corn silage, 8% hay, 30% corn grain, and 10% concentrate mix formulated to meet the nutritional requirements of a lactating dairy cow expected to produce 10,200 kg milk yr^{-1}. The IVTD (g kg^{-1} DM) and the *in vitro* digestibility of NDF (dNDF; g kg^{-1} aNDF) were calculated as below:

IVTD = [1 – (post digestion dry weight following aNDF wash/predigestion dry weight)] × 1000

dNDF = [1 – (post digestion dry weight following aNDF wash/predigestion dry weight of aNDF)] × 1000

The nutritive value attributes previously described were thereafter predicted for all forage samples using NIRS (WinISI III ver.1.61 software, Infrasoft International, LLC, Silver Spring, MD) and the statistics on the performance of these predictions are presented in Table 3. The NIRS predictions were considered successful when the Ratio of standard error of Prediction to standard Deviation (RPD) was greater than 3 (Nie et al., 2009). The RPD was calculated by dividing the standard deviation (SD) of the reference data used in the validation set by the standard error of prediction corrected for bias [SEP(C)]. Using the NIRS predicted values for B1+B2+B3+C, B2+B3+C, and B3+C of all forage samples, all protein fractions were then calculated (Licitra et al., 1996). The protein fraction A was calculated as the difference between the TN and the N insoluble in trichloroacetic acid (B1+B2+B3+C). The protein fraction B1 was calculated as the difference between N precipitable with trichloroacetic acid and insoluble N in a borate-phosphate buffer (B2+B3+C). The protein fraction B2 was calculated as the difference between the N insoluble in a borate-phosphate buffer and the N insoluble in neutral detergent (B3+C). The protein fraction B3 (the neutral detergent insoluble N potentially degradable) was obtained by the difference between the neutral detergent insoluble N and the acid detergent insoluble N (fraction C). All protein fractions were then expressed on a TN basis by multiplying the protein fraction by the TN concentration.

All forage samples were chemically analysed for DM and ash concentrations (LECO corporation, 2009). Crude fat was also determined in all forage samples using Ankom xt15 Extractor Technology Method (AOCS, 2003).

The CNCPS carbohydrate fractions (A, B1, B2, and C) were calculated according to Sniffen et al. (1992) and using the NIRS predicted values of the relevant nutritive value attributes. The carbohydrate fraction A (CA) includes soluble sugars and represents the carbohydrate fraction that is degraded rapidly in the rumen. Carbohydrate fraction B1 (CB1) has an intermediate rate of degradation and represents mainly starch and non-starch polysaccharides soluble in neutral detergent. Carbohydrate fraction B2 (CB2) is available cell wall and its degradation rate is slow, and carbohydrate fraction C (CC) represents unavailable cell wall and is undegradable and indigestible. Total carbohydrates (TC), expressed as g kg^{-1} DM, were calculated as follows: TC = 1000 – CP – crude fat – ash. The carbohydrate fractions were expressed as g kg^{-1} of TC and calculated as follow:

Structural carbohydrates = neutral detergent fiber (aNDF) – neutral detergent insoluble crude protein

Neutral detergent insoluble crude protein = neutral detergent insoluble N × 6.25

Non fiber carbohydrates = TC – Structural carbohydrates

Carbohydrate fraction A (CA) = Soluble sugars

Carbohydrate fraction B1 (CB1) = Non Fiber carbohydrates – soluble sugars

Carbohydrate fraction B2 (CB2) = Structural carbohydrates – carbohydrate fraction C

Carbohydrate fraction C (CC) = Lignin (ADL) × 2.4

Statistical analyses

Mean values of DM yield and laboratory analyses were assessed across treatments by analyses of variance (ANOVA) using the GENSTAT 14 statistical software package (VSN International, 2011). Treatments (legume-grass complex mixtures) were considered fixed effects while sites and replicates were considered random. Because the interest is in a measure of the population response and how it might change relative to the treatment factors, the two harvests were averaged to get the variance homogeneity and provide a more representative value for the mixtures. Differences were considered significant when $P < 0.05$. For each variate, the extreme high or low mean values among forage mixtures were identified after calculating an upper [overall mean + (2.86 × SEM / 2)] and a lower [overall mean – (2.86 × SEM / 2)] limit centered about the overall mean. A principal component analyses (PCA) was used to assess the relationships among variates (DM yield and nutritive attributes) and how variations in these variates are related to forage mixtures. The PCA was performed on the least squares means of the treatments using the correlation matrix method to give equal weight to all variates. The contribution of each variate to a principal component axis can be seen from its loadings (Fig. 1).

RESULTS AND DISCUSSION

Main Effects of Legumes and Grass Species mix

The two legume species did not affect DM yield, dNDF and protein fraction B3, soluble sugars, carbohydrate fraction A and protein fraction A concentrations of the eight complex mixtures but significantly affected the other evaluated nutritive value attributes (Table 4). Although the legume species have not significantly affected the DM yield, mixtures with alfalfa had numerically greater DM yield than mixtures with birdsfoot trefoil. Greater average DM yield for alfalfa than birdsfoot trefoil was reported by Lauriault et al. (2003), Smit et al. (2008), and Gierus et al. (2012) in binary mixtures. In the present experiment, mixtures with alfalfa had lower minimum plant density of legume and numerically greater minimum plant density of grass species than the average of the two legumes which may partly explain the numerically greater DM yield for alfalfa mixtures (Table 5).

Alfalfa-based complex mixtures had greater ADF and aNDF concentrations and lower IVTD while mixtures with birdsfoot trefoil had lower ADF and aNDF concentrations and greater IVTD than the average of the two legume species. The dNDF was similar for both legume species (Table 4). Alfalfa is known to have a greater NDF concentration than birdsfoot trefoil (Barker et al., 1999; Cassida et al., 2000; Gierus et al., 2012).

Alfalfa-based complex mixtures had greater starch concentration than the average of the 2 legume species and numerically greater soluble sugars concentration than mixtures with birdsfoot trefoil. Mixtures with birdsfoot trefoil, however, had lower concentrations of starch and TC but greater carbohydrate fraction B1 concentrations than the average of the two legume species.

The mixtures with birdsfoot trefoil had greater TN concentration and mixtures with alfalfa had lower TN concentration in comparison to the average of the two legume species. Julier et al. (2003) reported significantly greater CP concentration for birdsfoot trefoil than alfalfa. Mixtures with alfalfa had greater protein fraction C concentration than the average of the two legume species and this was due their greater ADF concentration. Alfalfa-based mixtures also had greater protein fraction B1 and lower protein fractions A and B2 concentrations while mixtures with birdsfoot trefoil had lower protein fractions B1 and C and greater protein fractions A and B2 concentrations than the average of the two legume species.

Grass species mixes significantly affected most of the nutritive value attributes measured in this experiment except TC, aNDF, carbohydrate fraction B2, starch, protein fraction A, carbohydrate fraction B1, and TN (Table 4). The grass species mixes with meadow fescue (TiMfRcKb and MfTiKb) had greater DM yield while the RcKbTfMb grass mix had lower DM yield than the average of the four grass species mixes. In agreement with our results, mixtures of white clover seeded with meadow fescue constituted one of the most productive mixture in an eastern Canada study (McKenzie et al., 2005).

The MfTiKb grass mix had lower ADF concentration and greater dNDF and IVTD, while the KbTfOrMb and RcKbTfMb grass mixes, both including with tall fescue, had lower dNDF than the average of the four grass species mixes. The KbTfOrMb grass mix had a greater ADF concentration and the RcKbTfMb grass mix had a lower IVTD than the average of the four grass species mixes. Skinner et al. (2004) reported high NDF concentration and low IVTD in complex mixtures with tall fescue grown in northeastern USA.

The MfTiKb grass mix had a greater concentration of starch and the TiMfRcKb grass mix had a greater soluble sugars concentration than the average of the four grass species mixes. Because of the greater soluble sugars concentration, the TiMfRcKb grass mix also had the greater carbohydrate fraction A concentration.

The RcKbTfMb grass mix had a greater TN concentration compared to the average of the four grass species mixes, which can be due the presence of Mb, Kb, and Rc in this mix. This grass mix also had a greater minimum plant density of Kentucky bluegrass and reed canarygrass while the TiMfRcKb grass mix had a lower TN concentration and a lower minimum plant density of Kentucky bluegrass than the average of four grass species mixes (Table 5). Pelletier et al. (2010) reported greater TN concentrations in meadow bromegrass, Kentucky bluegrass, and reed canarygrass than in timothy and tall fescue forages when grown alone.

Comparison of the eight complex mixtures

The legume × grass species mix interaction was not significant for DM yield nor for nutritive value attributes, except for ADF and carbohydrate fraction A concentrations (Table 4). The ATiMfRcKb complex mixture had the greatest DM yield (Table 7) even though it was not significantly greater than the DM yield of the other complex mixture (Table 4).

A PCA was performed to determine the best complex mixture for DM yield and most of the nutritive value attributes. The PCA also provides information on the relationships among the nutritive value attributes. The PCA explained 82% of the total variation, with 67% on the first component and 15% on the second component (Fig. 1). The first component was largely defined by TC, aNDF, protein fraction C, carbohydrate fraction B2, starch, DM yield, protein fraction B3, and protein fraction B1 on the positive side, and by TN, carbohydrate fraction B1, carbohydrate fraction C, IVTD, protein fractions A, and B2 on the negative side. Variates within the same group on each side were positively correlated while variates in opposing groups were negatively correlated. Thus, mixtures with high DM yield tended to have high concentrations of TC, aNDF, protein fraction C, carbohydrate fraction B2, starch, ADF, protein fractions B3, and B1 and low IVTD, TN, carbohydrates fractions B1 and C, protein fractions A, and B2 concentrations. The second component of the PCA defined a contrast dominated on the positive side by carbohydrate fraction A, dNDF, and soluble sugars (Fig. 1). On the negative side, it was largely determined by ADF. Increased concentrations of soluble carbohydrates and starch in high-NSC versus low-NSC alfalfa were associated with lower concentrations of NDF, ADF, and CP in forages grown in Québec, Canada (Berthiaume et al., 2010).

The first component of the PCA mostly defined differences among legume species, separating the mixtures with birdsfoot trefoil on the left (negative), with high TN concentrations and low DM yield, from mixtures with alfalfa on the right (positive), with high DM yield and aNDF concentrations (Fig. 1). The legume had a greater influence than the grass species on the nutritive value and DM yield of the mixtures evaluated in this experiment. The first principal component was responsible for most of the total variation (67%) and it was strongly determined by the legume species. The second component defined differences among the four grass species mixes, separating the TiMfRcKb and MfTiKb mixes, both including timothy and meadow fescue, from the others two grass species mixes. Thus, AMfTiKb and

ATiMfRcKb mixtures had greater carbohydrate fraction A concentration and dNDF than ARcKbTfMb and AKbTfOrMb complex mixtures (Y axis in figure 1). The Birdsfoot trefoil mixtures with timothy and meadow fescue showed the same trend but at a reduced level.

The AMfTiKb complex mixture had a large first PC score (Table 7) and consequently associated with great TC, aNDF, protein fraction C, carbohydrate fraction B2, DM yield, ADF, protein fraction B3, soluble sugars, and carbohydrate fraction A. This complex mixture presented the best combination of high concentration of readily available sugars, WSC/CP balance, dNDF, and DM yield.

Energy/protein balance

The extent to which soluble N is utilized in the rumen depends on the rate of release and the concentration of carbohydrates and N availability (Hristov et al., 2005). For improved N utilization, it is suggested to have a combination of high energy availability for microbial proteins, reduced total N concentration or a reduction in N solubility (Bryant et al., 2012). Thus, the ratios between WSC and N and between readily fermentable carbohydrates (carbohydrate fractions A+B1) and soluble N (protein fractions A+B1) are important determinants of ruminal N losses. Lee et al. (2002) and Hristov et al. (2005) reported reduction of rumen ammonia when more fermentable sugars were available in the rumen.

Edwards et al. (2007) reported that the dietary WSC/CP ratio should be above 0.70 to provide better use of dietary N for milk production and reduced N losses in urine. In the present study, the alfalfa-based complex mixture showed significantly greater WSC/CP ratio with lower concentrations of TN and protein fraction A and greater concentration of TC. Thus, the mixtures with alfalfa also showed the best (carbohydrate fractions A+B1)/(protein fractions A+B1) ratio (Table 8).

The TiMfRcKb grass mix had greater WSC/CP and (carbohydrate fractions B1+A)/(protein fractions B1+PA) ratios than the average of the 4 grass species mixes (Table 8). In comparison to the average of the 8 complex mixtures, the ATiMfRcKb complex mixture showed a greater WSC/CP ratio followed by AMfTiKb. The ATiMfRcKb complex mixture also had great carbohydrate fraction A and TC concentrations, low TN concentration, and great (carbohydrate fractions A+B1)/(protein fractions A+B1) ratio (Table 9).

Our results from first two harvests at two sites apply to the first production year in which all seeded species were present following a successful establishment (Tables 5 and 6). The persistence of some of those species, particularly under frequent cuttings, might not be sufficient to sustain production over several years. In Pennsylvania State (USA), it was observed that the number of species seeded in mixtures declined by 30% from the first to the third production year (Tracy and Sanderson, 2004). In eastern Canada, frequent cutting has been shown to reduce the persistence of alfalfa (Dhont et al., 2004), tall fescue (Drapeau et al., 2007), and meadow fescue (Drapeau and Bélanger, 2009).

CONCLUSIONS

The timothy, meadow fescue, reed canarygrass and Kentucky bluegrass grass species mix provided the best combination of great readily-available energy to protein balance, great DM yield, and average digestibility. The complex mixtures with alfalfa and meadow fescue had the best readily-available energy to protein ratio and DM yield. However, alfalfa-based complex mixtures had lower digestibility than birdsfoot trefoil based mixtures. The complex mixtures with birdsfoot trefoil, however, had greater digestibility and lower readily-available energy to protein balance. These results from the first two harvests of the first production year provide useful and novel information on the desired species composition of complex mixtures that combine high readily-available energy to protein ratio and DM yield. Further research is needed to determine the feasibility of maintaining this desired composition throughout the growing season and over several cropping years.

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FIGURE CAPTION

Figure 1. Diagram of the first two principal component scores calculated for each of the eight complex mixtures (■) and for each nutritive value attribute (●) to illustrate the relationship among DM yield and carbohydrate and protein fractions, and among the complex mixtures. PC1 = First principal component score; PC2 = Second principal component score; TC = total carbohydrates; aNDF = neutral detergent fibre using α-amilase; PC = bound protein undegraded in the rumen and indigestible in the intestine; CB2 = carbohydrates compounds slowly degraded; ST = starch; DMY = dry matter yield ; ADF = acid detergent fibre; PB3 = slowly degraded protein; PB1 = rapidly degraded protein; SS = soluble sugars; CA = carbohydrate compounds instantaneously degraded in the rumen; dNDF = *in vitro* digestibility of NDF; PB2 = protein with intermediate rate of ruminal degradation; PA = non protein nitrogen compounds instantaneously degraded in the rumen; IVTD = *in vitro* true digestibility of DM; CC = unavailable fiber; CB1 = carbohydrate compounds intermediately degraded; and TN = total nitrogen. A = Alfalfa; B = Birdsfoot trefoil; Ti = Timothy; Kb = Kentucky bluegrass; Tf = Tall fescue; Or = Orchardgrass; Mf = Meadow fescue; Mb = Meadow bromegrass; Rc = reed canarygrass.

Table 1. Average monthly precipitation and accumulation of degree-days along with the monthly 30-yr average (1971-200) for both experimental sites.

		Precipitation (mm)		**Degree-days (ºC)†**	
Site	**Month**	**2011**	**30-yr avg.**	**2011**	**30-yr avg.**
Levis‡	May	130	106	183	199
	June	87	114	354	343
Normandin§	May	96	85	132	145
	June	68	78	303	281

†Degree-days were calculated on 5ºC basis.

‡Reported data were taken at the Quebec airport (46º48 `N, 71º23 `W, and elevation 74 m) (Environment Canada, 2011).

§Reported data were taken at Normandin (48º51` N, 72º32` W, and elevation 137 m) (Environment Canada, 2011).

Table 2. Stages of development of the legume and grass species for the first two harvests from successive regrowths at Lévis and Normandin.

Species	**Lévis**		**Normandin**	
	Harvest 1 (02 June)	**Haverst 2 (22 June)**	**Harvest 1 (06 June)**	**Harvest 2 (27 June)**
Alfalfa	Late vegetative	Early bud	Mid vegetative	Late vegetative
Birdsfoot trefoil	Early bud	Late bud	Early bud	Late bud
Orchardgrass	Booting (45)†	Tillering (21)	Stem elongation	Inflorescence emergency (50)
Timothy	Stem elongation (31)	Inflorescence emergence (56)	Stem elongation	Inflorescence emergence (56)
Kentucky bluegrass	Inflorescence emergency (54)	Anthesis (60)	Tillering (20)	inflorescence emergence (56)
Tall fescue	Stem elongation (31)	Anthesis (60)	Stem elongation	inflorescence emergence (58)
Meadow bromegrass	Stem elongation (31)	Stem elongation (31)	Stem elongation	Stem elongation (39)
Meadow fescue	Booting (45)	Anthesis (60)	Stem elongation	inflorescence emergence (58)
Reed canarygrass	Stem elongation (32)	Stem elongation (32)	Stem elongation	Stem elongation (37)

†Stages of development of the grasses were determined according to Simon and Park (1981): 20, no elongated sheath; 21,1 elongated sheath; 22, 2 elongated sheaths; 31, first node palpable; 32, second node palpable; 37, flag leaf just visible; 39, flag leaf ligule/collar just visible; 45, boot swollen; 50, upper 1 to 2 cm of inflorescence visible;54, ½ of inflorescence emerged; 56, ¾ of inflorescence emerged; 58, base of inflorescence just visible; 60, pre-anthesis.

Table 3. Statistics on the performance of near infrared spectroscopy (NIRS) to predict the nutritive value attributes of the validation set of samples.

Constituent†	**n‡**	**Slope**	**Mean**	**SD**	**SEP**	**RSQ**	**SEP(C)**	**RPD**
ADF (g kg^{-1} DM)	15	0.94	291	35	10	0.94	9.1	3.9
aNDF (g kg^{-1} DM)	15	1.01	416	66	15	0.96	13	5.1
ADL (g kg^{-1} DM)	14	0.99	30	8.1	2.0	0.94	2.0	4.1
TN (g kg^{-1} DM)	15	1.00	31	5.1	0.79	0.98	0. 8	6.5
Protein fractions								
B1+B2+B3+C (g TN kg^{-1} DM)	15	1.02	24	3.2	0.84	0.94	0.8	4.0
B2+B3+C (g TN kg^{-1} DM)	15	1.00	21	4.2	0.80	0.96	0.8	5.1
B3+C (g TN kg^{-1} DM)	15	1.10	6.8	1.97	0.46	0.95	0.47	4.2
C (g TN kg^{-1} DM)	14	0.93	1.2	0.36	0.15	0.81	0.16	2.3
Starch (g kg^{-1} DM)	15	0.95	34	32	8.91	0.93	8.7	3.7
Soluble sugars (g kg^{-1} DM)	15	0.91	103	30	10	0.92	9.0	3.3
IVTD (g kg^{-1} DM)	15	0.02	910	21	7.4	0.90	6.6	3.2
dNDF (g kg^{-1} NDF)	15	0.99	797	26	7.7	0.93	7.1	3.7

†ADF = acid detergent fiber; aNDF = neutral detergent fiber using α-amylase; ADL = acid detergent lignin; TN = total nitrogen; B1 = rapidly degraded protein; B2 = protein with intermediate rate of ruminal degradation; B3 = slowly degraded protein; C = bound protein undegraded in the rumen and indigestible in the intestine = nitrogen insoluble in acid detergent, B1+B2+B3+C = true protein precipitated with tungstic acid, B2+B3+C = nitrogen insoluble in a borate-phosphate buffer, B3+C = nitrogen insoluble in neutral detergent, IVTD = *in vitro* true digestibility of dry matter, and dNDF = *in vitro* digestibility of NDF.

‡n = number of samples in the validation set; SD = standard deviation; SEP = standard error of prediction; SEP(C) = standard error of prediction corrected for the bias; RSQ = coefficient of determination for the prediction; RPD = ratio of prediction to deviation (SD of the validation set of samples/SEP(C)).

Table 4. Nutritive value attribute means and DM yield for the main effects of two legumes and four grass species mixes. Nutritive attributes and DM yield were sorted according to the first principal component scores†. Values are the average of the first two harvests at two sites.

Main effects	TC‡	NDF	PC	CB2	ST	DMY	ADF	PB3	PB1	SS	CA	dNDF	PB2	PA	IVTD	CC	CB1	TN
	g kg^{-1} DM		g kg^{-1}TN	g kg^{-1} TC	g kg^{-1} DM	Mg ha^{-1}	g kg^{-1} DM	g kg^{-1} TN		g kg^{-1} DM	g kg^{-1} TC	g kg^{-1} NDF	g kg^{-1} TN		g kg^{-1} DM	g kg^{-1} TC		g kg^{-1} DM
Legumes																		
Birdsfoot trefoil	704	452	38.8	491	31.6	1.26	295	170	129	128	181	799	**426**	**236**	**913**	**91.8**	**235**	**30.1**
Alfalfa	**745**	**486**	**44.7**	**528**	**40.0**	1.36	**312**	**178**	**145**	138	184	799	408	225	902	76.4	208	24.9
SEM¶	5.51	3.9	0.87	4.44	2.17	0.10	2.78	3.15	5.05	4.93	5.61	2.58	4.36	3.95	1.39	2.06	5.77	0.55
upper limit	732	475	43	515	38.9	1.45	307	178	144	140	191	803	423	236	909	87.0	230	28.3
lower limit	717	463	41	503	33.0	1.17	299	169	129	126	174	795	411	225	905	81.0	214	27.0
Grass mixes§																		
TiMfRcKb	727	473	**42.6**	511	34.8	**1.37**	304	**176**	134	**139**	**191**	**802**	419	228	907	82.1	214	26.9
KbTfOrMb	722	**474**	**43.0**	513	34.0	1.30	**308**	**177**	**143**	123	171	796	408	230	907	**88.0**	228	27.9
MfTiKb	728	465	**43.0**	502	**38.7**	**1.34**	299	175	**146**	135	186	**804**	410	225	**910**	85.1	223	27.3
RcKbTfMb	720	465	38.5	511	35.7	1.22	302	167	124	134	183	794	**431**	**240**	906	81.1	222	**28.0**
SEM¶	3.11	3.65	0.59	4.91	1.52	0.02	2.06	1.61	4.17	2.75	3.14	1.67	2.54	4.06	0.97	1.46	5.10	0.31
upper limit	729	474	42.6	516	38.0	1.34	306	176	143	137	187	801	421	237	909	86.2	229	28.0
lower limit	720	464	40.9	502	33.6	1.27	300	172	131	129	178	797	413	225	906	82.0	215	27.1
Overall mean	725	469	41.8	509	35.8	1.31	303	174	137	133	183	799	417	231	907	84.1	222	27.5

Sources of variation	df#	*P* value																
Legumes (L)	1	***	***	***	***	*	ns	***	ns	*	ns	ns	ns	*	ns	***	***	**	***
Grass mixes (G)	3	ns	ns	***	ns	ns	***	*	***	**	**	***	***	***	ns	*	**	ns	ns
L × G	3	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns

† For each attribute, the extreme high and low values among forage mixtures are noted in bold or underlined, respectively, after calculating an upper [overall mean + (2.86 × SEM / 2)] and a lower [overall mean – (2.86 × SEM / 2)] limit, centered about the overall mean.

‡TC = total carbohydrates ; PC = bound protein undegraded in the rumen and indigestible in the intestine; aNDF = neutral detergent fibre using α-amilase; ST = starch; DMY = dry matter yield ; CB2 = carbohydrate compounds slowly degraded; ADF = acid detergent fibre; PB3 = slowly degraded protein; PB1 = rapidly degraded protein; SS = soluble sugars; CA = carbohydrate compounds instantaneously degraded in the rumen; dNDF = *in vitro* digestibility of NDF; CB1 = carbohydrate compounds intermediately degraded; PB2 = protein with intermediate rate of ruminal degradation; PA = non protein nitrogen compounds instantaneously degraded in the rumen; IVTD = *in vitro* true digestibility of DM; CC = unavailable fiber; and TN = total nitrogen.

§where Ti = Timothy, Kb = Kentucky bluegrass, Tf = Tall fescue, Or = Orchardgrass, Mf = Meadow fescue, Mb = Meadow bromegrass, Rc = reed canarygrass

¶SEM = Standard error of the mean
#Degrees of freedom.

Table 5. Minimum plant density (plant m^{-2})† of each species after the first harvest of the first production year. Values are the average of two sites‡.

Main effects		**Legumes**	**Other species**	**Rc#**	**Kb**	**Tf**	**Mb**	**Or**	**Ti**	**Mf**
Legumes	Birdsfoot	**37.2**	17.2	7.03	7.26	16.7	6.95	5.33	8.85	17.7
	Alfalfa	28.9	**19.1**	7.70	9.82	17.1	6.43	6.12	8.87	17.7
	SEM§	0.71	0.62	0.54	0.92	0.28	0.50	0.33	0.70	0.28
	upper limit	34.0	19.0	8.13	9.85	17.3	7.4	6.19	9.86	18.1
Grass mixes	lower limit	32.0	17.2	6.59	7.23	16.5	6.0	5.26	7.86	17.3
	TiMfRcKb#	32.9	16.6	**13.05**	7.40	-	-	-	**16.1**	**33.6**
	KbTfOrMb	31.1	18.1	-	7.15	**33.4**	**14.35**	**22.9**	-	-
	MfTiKb	33.5	18.3	-	9.05	-	-	-	**19.4**	**37.2**
	RcKbTfMb	**34.7**	**19.6**	**16.4**	**10.55**	**34.1**	**12.4**	-	-	-
	SEM	0.56	0.72	0.59	0.59	0.38	0.61	0.51	0.74	0.45
	upper limit	33.8	19.1	8.20	9.38	17.3	7.57	6.46	9.92	18.3
	lower limit	32.2	17.1	6.52	7.70	16.3	5.83	5.00	7.80	17.0
Overall mean		33	18.1	7.36	8.54	16.9	6.69	5.73	8.86	17.7
Source variation	df¶	*P* value								
Legumes (L)	1	***	ns	ns	ns	ns	ns	ns	ns	ns
Grass mixes (G)	3	***	*	***	***	***	***	***	***	***
L × G	3	ns	ns	ns	ns	**	ns	ns	ns	ns

†Determined as described by Vogel and Masters (2001).

‡For each variate, the extreme high and low values within legume and grass species mix are noted in bold and underline, respectively, after calculating an upper [overall mean + (2.86 × SEM / 2)] and a lower [overall mean - (2.86 × SEM / 2)] limit, centered on the overall mean.

#Ti = Timothy, Kb = Kentucky bluegrass, Tf = Tall fescue, Or = Orchardgrass, Mf = Meadow fescue, Mb = Meadow bromegrass, Rc = reed canarygrass.

§Standard error of the Mean.

¶Degrees of freedom.

*** significant at $P < 0.001$, ** significant at $P < 0.01$, * significant at $P < 0.05$.

Table 6. Minimum plant density (plant m^{-2})† of each species in each complex mixture after the first harvest of the first production year. Values are the average of two sites‡.

	Forage mixtures#											
	BTiMfRgKb	**BKbTfOrMb**	**BMfTiKb**	**BRcKgTfMb**	**ATiMfRgKb**	**AKbTfOrMb**	**AMfTiKb**	**ARcKbTfMb**	**Mean**	**SEM§**	**Upper limit**	**Lower limit**
Legumes	**37.5**	34.3	**38**	**39**	28	27.9	29	30.4	33.0	0.98	34.4	31.6
Grass Species												
Reed canarygrass	**13.8**	–	–	**14.3**	**12.3**	–	–	18.5	7.36	0.9	8.65	6.07
Kentucky bluegrass	6.1	6.5	7.2	9.2	8.7	7.8	**10.9**	**11.9**	8.54	1.17	10.21	6.87
Tall fescue	–	**31.8**	–	**34.8**	–	**35**	–	**33.4**	16.9	0.55	17.7	16.11
Meadow brome	–	**14.8**	–	**13.0**	–	**13.9**	–	**11.8**	6.69	0.9	7.98	5.40
Orchardgrass	–	**21.3**	–	–	–	**24.5**	–	–	5.73	0.71	6.74	4.72
Timothy	**15.5**	–	**19.9**	–	**16.6**	–	**18.9**	–	8.86	1.14	10.49	7.23
Meadow fescue	**33.5**	–	**37.4**	–	**33.6**	–	**37**	–	17.7	0.61	18.57	16.83
Other Species	15.01	17.4	16.6	**19.8**	18.2	18.8	**19.9**	19.4	18.1	1.1	19.67	16.53

†Determined as described by Vogel and Masters (2001).

‡For each variate, the extreme high and low values within legume and grass species mix are noted in bold and underline, respectively, after calculating an upper [overall mean + (2.86 ×SEM / 2)] and a lower [overall mean - (2.86 ×SEM / 2)] limit, centered on the overall mean.

#where B = birdsfoot trefoil, A = alfalfa , Ti = timothy, Mf = meadow fescue, Rc = reed canarygrass, Kb = Kentucky bluegrass, Tf = tall fescue, Or = orchardgrass, and Mb = meadow bromegrass.

§Standard error of the mean.

Table 7. The two principal component scores and means of all nutritive value attributes and DM yield of 8 complex mixtures sampled at the first two harvests and at two sites. The complex legume-grass mixtures and nutritive value attributes and DM yield were sorted according to the first principal component scores†. Values are the average of the first two harvests at two sites.

Complex	**PC1**	**PC2**	**TC**	**aNDF**	**PC**	**CB2**	**ST**	**DMY**	**ADF**	**PB3**	**PB1**	**SS**	**CA**	**dNDF**	**PB2**	**PA**	**IVTD**	**CC**	**CB1**	**TN**
mixtures	(λ_1 = 67%)	(λ_2 = 15%)	g kg^{-1} DM	g kg^{-1} DM	g kg^{-1} TN	g kg^{-1} TC	g kg^{-1} DM	Mg ha^{-1}	g kg^{-1} DM	g kg^{-1} TN		g kg^{-1} DM	g kg^{-1} TC	g kg^{-1} NDF	g kg^{-1} TN		g kg^{-1} DM	g kg^{-1} TC		g kg^{-1} DM
ATiMfRcKb§	**4.16**	1.37	**752**	**492**	**45.5**	**532**	38.2	**1.45**	**313**	**180**	138	**147**	**197**	803	414	223	901	74.6	196	24.1
AMfTiKb	**3.68**	**2.01**	**744**	477	**46.0**	516	**44.7**	1.39	301	**180**	**154**	**144**	**194**	**804**	403	217	906	77.2	204	25.0
AKbTfOrMb	**3.62**	-2.35	**743**	**495**	**46.1**	**538**	37.9	1.37	**321**	**181**	**155**	125	169	797	394	225	900	79.0	214	24.9
ARcKbTfMb	0.90	-2.24	**739**	**481**	41.2	**525**	39.4	1.25	**312**	169	131	135	176	791	421	237	900	74.8	218	25.7
BTiMfRcKb	-2.43	1.05	702	454	39.7	491	31.5	1.29	295	173	129	131	186	801	**425**	233	**913**	**89.6**	231	**29.7**
BMfTiKb	-2.44	0.61	712	453	39.9	488	32.8	1.30	297	170	139	126	177	**805**	418	234	**914**	**93.0**	**241**	**29.7**
BKbTfOrMb	-3.51	-0.78	702	452	39.9	489	30.1	1.23	294	173	131	121	172	796	422	235	**913**	**97.0**	**242**	**30.8**
BRcKbTfMb	-3.98	0.32	701	449	35.9	497	32.0	1.20	293	164	116	133	190	796	**441**	**243**	**912**	87.5	226	**30.2**
Overall mean	0	0	725	469	41.8	509	35.8	1.31	303	174	137	133	183	799	417	231	907	84.1	222	27.5
SEM¶	1	1	6.69	5.94	1.13	7.48	2.86	0.10	3.76	3.72	7.19	5.97	6.79	3.29	5.36	6.35	1.83	2.73	8.5	0.67
upper limit	1.43	1.43	734	478	43.4	520	39.9	1.45	309	179	147	141	192	804	425	240	910	88.0	234	28.5
lower limit	-1.43	-1.43	715	460	40.2	498	31.7	1.17	298	169	126	124	173	794	409	222	905	80.2	210	26.6

† For each attribute, the extreme high and low values among forage mixtures are noted in bold or underlined, respectively, after calculating an upper [overall mean + (2.86 × SEM / 2)] and a lower [overall mean – (2.86 × SEM / 2)] limit, centered about the overall mean.

‡TC = total carbohydrates; aNDF = neutral detergent fibre using α-amilase; PC = bound protein undegraded in the rumen and indigestible in the intestine; CB2 = carbohydrates compounds slowly degraded; ST = starch; DMY = dry matter yield ; ADF = acid detergent fibre; PB3 = slowly degraded protein; PB1 = rapidly degraded protein; SS = soluble sugars; CA = carbohydrate compounds instantaneously degraded in the rumen; dNDF = *in vitro* digestibility of NDF; CB1 = carbohydrate compounds intermediately degraded; PB2 = protein with intermediate rate of ruminal degradation; PA = non protein nitrogen compounds instantaneously degraded in the rumen; IVTD = *in vitro* true digestibility of DM; CC = unavailable fiber; and TN = total nitrogen.

§B = Birdsfoot trefoil, A = Alfalfa, Ti = Timothy, Kb = Kentucky bluegrass, Tf = Tall fescue, Or = Orchardgrass, Mf = Meadow fescue, Mb = Meadow bromegrass, and Rc = reed canarygrass.

¶SEM = Standard error of the mean.

Table 8. Ratios of WSC/CP and (CA+CB1)/(PA+PB1)† for the main effects of two legume species and four grass species mixes. Values are the average of the first two harvests at two sites‡.

Main effects		g WSC g^{-1} CP	g (CA+CB1) g^{-1} (PA+PB1)
Legumes			
Birdsfoot trefoil		0.69	4.47
Alfalfa		**0.94**	**5.42**
SEM¶		0.07	0.25
upper limit		0.91	5.30
lower limit			
Grass mixes		0.72	4.60
TiMfRcKb‡		**0.87**	**5.08**
KbTfOrMb		0.74	4.78
MfTiKb		0.84	4.96
RcKbTfMb		0.82	4.97
SEM		0.03	0.08
upper limit		0.86	5.07
lower limit		0.78	4.83
Overall mean		0.82	4.95
Sources of variation	df	*P* value	
Legumes (L)	1	*	*
Grass mixes (G)	3	*	ns
L × G	3	ns	ns

†CA = carbohydrate compounds instantaneously degraded in the rumen; CB1 = carbohydrate compounds intermediately degraded; CP = crude protein; PA = non-protein nitrogen compounds instantaneously degraded in the rumen; PB1 = rapidly degraded protein; WSC, water soluble carbohydrates.

‡For each variate, within legume and grass species mix the extreme high and low values are noted in bold or underlined, respectively, after calculating an upper [overall mean + (2.86 × SEM / 2)] and a lower [overall mean – (2.86 × SEM / 2)] limit, centered about the overall mean.

¶SEM = Standard error of the mean.

‡where Ti = Timothy, Kb = Kentucky bluegrass, Tf = Tall fescue, Or = Orchardgrass, Mf = Meadow fescue, Mb = Meadow bromegrass, and Rc = reed canarygrass.

ns = not significant, * = Significant at $P < 0.05$.

Table 9. Ratios of WSC/CP and (CA+CB1)/(PA+PB1)† for the 8 complex legume-grass mixtures. Values are the average of two harvests at two sites‡.

Complex mixtures§	g WSC g^{-1} CP	g (CA+CB1) g^{-1} (PA+PB1)
ATiMfRcKb	**1.04**	**5.64**
AMfTiKb	**0.98**	**5.43**
AKbTfOrMb	0.84	5.22
ARcKbTfMb	0.89	**5.40**
BTiMfRcKb	0.71	4.51
BMfTiKb	0.69	4.50
BKbTfOrMb	0.64	4.33
BRcKbTfMb	0.74	4.55
Mean	0.82	4.95
SEM¶	0.07	0.27
upper limit	0.92	5.33
lower limit	0.71	4.57

†CA = carbohydrate compounds instantaneously degraded in the rumen; CB1 = carbohydrate compounds intermediately degraded; CP = crude protein; PA = non-protein nitrogen compounds instantaneously degraded in the rumen; PB1 = rapidly degraded protein; WSC, water soluble carbohydrates.

‡For each variate, among forage mixtures the extreme high and low values are noted in bold or underlined, respectively, after calculating an upper [overall mean + (2.86 × SEM / 2)] and a lower [overall mean – (2.86 × SEM / 2)] limit, centered about the overall mean.

§where B = Birdsfoot trefoil, A = Alfalfa, Ti = Timothy, Kb = Kentucky bluegrass, Tf = Tall fescue, Or = Orchardgrass, Mf = Meadow fescue, Mb = Meadow bromegrass, and Rc = reed canarygrass.

¶SEM = Standard error of the mean.

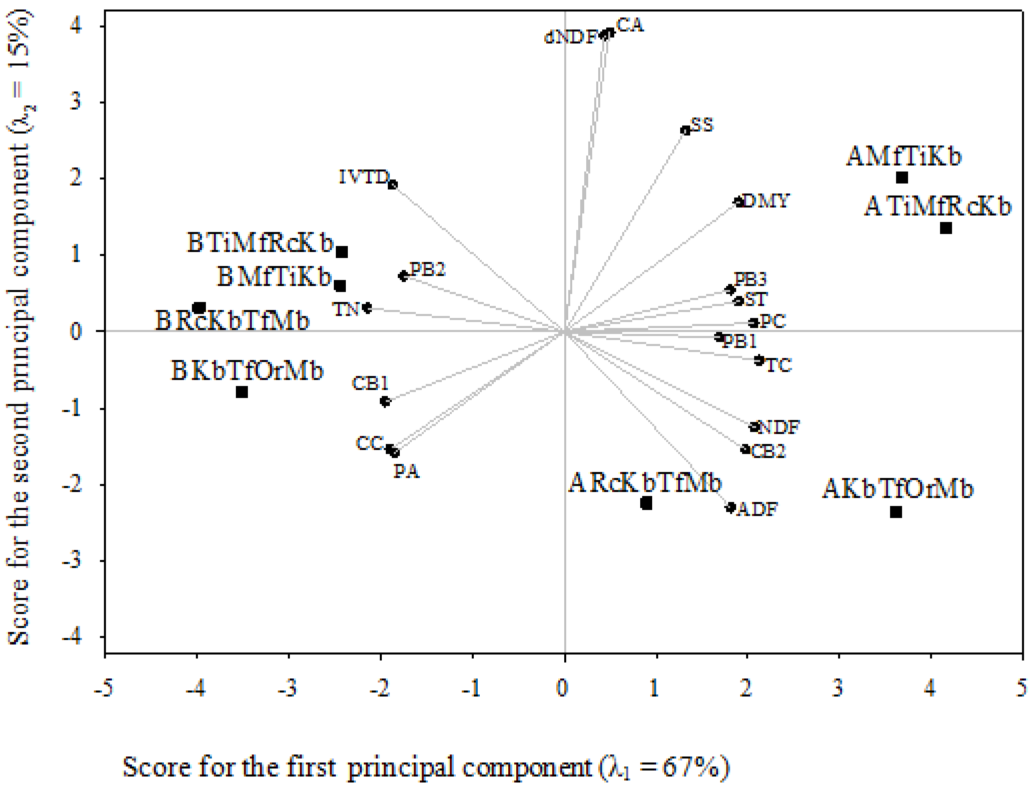
Score for the second principal component (λ_2 = 15%)
4
3
2
1
0
-1
-2
-3
-4
dNDF
CA
SS
AMfTiKb
IVTD
DMY
ATiMfRcKb
BTiMfRcKb
BMfTiKb
PB2
PB3
ST
TN
BRcKbTfMb
PC
PB1
TC
BKbTfOrMb
CB1
NDF
CC
CB2
PA
ARcKbTfMb
AKbTfOrMb
ADF
-5
-4
-3
-2
-1
0
1
2
3
4
5
Score for the first principal component (λ_1 = 67%)

Fig. 1

CONSIDERAÇÕES FINAIS

Entre as gramíneas avaliadas nos experimentos 1 e 2, a meadow fescue demonstrou ter maior potencial para produção. Em ambos os experimentos as misturas de alfafa com meadow fescue foram as melhores em produção e na relação energia proteína. Misturas com alfafa tiveram as mais baixas digestibilidades e as mais baixas concentrações de N.

O primeiro componente da Análise do Componente Principal (PCA) no experimento 1 definiu as diferenças entre as espécies de gramíneas nas misturas, enquanto o segundo componente definiu as diferenças entre as leguminosas existentes nestas misturas. Já no experimento 2, ocorreu o inverso. O primeiro componente definiu as diferenças entre as leguminosas e o segundo componente as diferenças entre as gramíneas nas misturas avaliadas. No entanto, não houve diferença entre os dois experimentos para as correlações entre alguns atributos de valor nutricional feitas pelo PCA. Em ambos experimentos a produção de matéria seca foi positivamente correlacionada com as concentrações de FDA e FDN e negativamente correlacionada com a digestibilidade e as concentrações de N.

Os resultados obtidos nos dois experimentos contribuíram com informações relevantes das combinações entre as espécies de gramíneas e leguminosas, principalmente no que diz respeito a produção e ao balanço energético e na combinação de ambos. No entanto, não se pode ignorar a necessidade de investigações futuras para avaliar estas misturas a longo prazo. Cientes desta necessidade estes experimentos foram implantados e desenvolvidos também com o intuito de avaliar as características de valor nutritivo e de persistência destas misturas em vários anos.

A determinação das taxas de degradação ruminal podem auxiliar a estimar, de forma ponderada, a sincronia de utilização de frações solúveis de carboidratos e proteínas pelos microrganismos do rúmen em intervalos de tempo. Desta forma, sugere-se estudos neste sentido.